PATHOLOGICAL STUDIES OF SOME FRUITS AND VEGETABLES INFECTED BY FUNGI IN MARKETS

THESIS

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By

MRS. RAFAT RAZA

M.Sc. (Botany)

DEPARTMENT OF BOTANY

UNIVERSITY OF ALLAHABAD-211002

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- Dedicated to -

My Uncle **Mr. Nazir Rizvi**

the greatest inspirational source

who made this thesis possible.

CERTIFICATE

*Certified that the present research work has been carried out by **Mrs. Rafat Raza** under the supervision of Late Dr. Virendra Bhargava, Reader in Botany Department and Prof. B.Lal, Ex. Head Botany Department, Allahabad University. She has submitted the thesis under my supervision.*

(D.N. Shukla)
M.Sc.(Ag.) Bot., D.Phil,
D.Sc.
(Supervisor)

Department of Botany
University of Allahabad
Allahabad-211002

PREFACE

The thesis embodies the work done by me on the topic "**Pathological studies of some fruits and Vegetables infected by fungi in markets**". The work was done in the 'Sadasivan, Myco-Pathology Laboratory' of Botany Department, University of Allahabad.

The whole work has been presented in the form of number of Chapters. There are in all 10 main chapters. The first one includes the general introduction. The second chapter incorporates the materials used and the methods employed during the investigation period. Chapter three deals with all the fungi that were isolated from different hosts. Chapter four provides the information regarding the nature of disease, symptom, etc. The effect of environment on disease development is discussed in chapter five and economic losses and the chemical analysis, effect of temperature, pH has been given in the chapter six. The possible suitable control measures are given in chapter seven. Chapter eight incorporates the Discussion and conclusion based on the results of the

investigations. A brief summary of the whole work, has been given in the ninth chapter.

The literature on various aspects of post-harvest diseases is enormous and expanding rapidly. In most of the cases, only those references have been cited which are comparatively more recent. The bibliography of references cited in this thesis is given in the end.

Sadasivan Mycopathological Laboratory,
Department of Botany,
University of Allahabad,
Allahabad-211002.

Rafat
[RAFAT RAZA]
M.Sc.(Botany)

Dated :

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And last but not the least, I owe my success to my husband, Mr. F.A. Hashmi and my parents without whose blessings, encouragement, financial and morale support it would not have been possible for me to complete my thesis work.

I would be failing in my duty if I don't acknowledge the patience of my son, Sahil during the execution of this work.

Rafat

(RAFAT RAZA)
M.Sc. (Botany)

Department of Botany
University of Allahabad
Allahabad-211002

Dated : 30.12.2002

CONTENTS

INTRODUCTION

The individual who ranks fortune ahead of fame, gravitates into business where profit is the payoff. Those who value fame ahead of fortune go into science where publishing and fame are the payoffs.

[Anonymous]

"**Vegetable** and **herbs** are scientifically defined as those plants that produce edible structures that are not reproductive in nature, which means, that we eat the stems and leaves not the fruit." However, this definition does not follows the public's distinction between what is considered a fruit and what is considered a vegetable. Using this definition, tomatoes and peppers are considered fruit but most people refer to them as vegetables. For our purposes, we will use the public's perception of these plants to distinguish between them. Vegetables and herbs are those plants that produce edible structures and are grown as annual crops. Vegetables by this definition

include tomatoes, peppers and pumpkins and herbs include plants such as rosemary, basil and thyme.

"**Fruit** crops are scientifically defined as those plants that produce edible reproductive structures". However, this definition does not follow the public's distinction between what is considered a fruit and what is considered a vegetable. Using this definition, tomatoes and peppers are considered fruit but most people refer to then as vegetables. For our purposes, we will use the public's perception of these plants to distinguish between them. Fruits are those plants that produce edible reproductive structures and are grown as perennial crops Fruits include tree fruits such as apple, pear, peach, etc. and small fruits crops such as raspberry, blackberry, gooseberry, etc.

The first necessity of human beings is food. The primitive man obtained his food from wild plants. But with the advancement of civilization, the man-started cultivation of plants to meet the requirements of his food. The available evidences indicate the

domestication of plants dates back to 7,000 B.C. The prehistoric man discovered the virtue of certain wild plants and profoundly altered them into useful cultivated plants.

Earlier Greek and Roman naturalists like **Theophrastus**, **Dioscorides**, **Pliny** the Elder and **Galen** laid down the scientific foundation of domestication of wild plants. Later, **Darwin's** evolutionary theory (1868) suggested that origin of useful cultivated plants has occurred through natural selection and hybridisation.

Cultivated plants are a product of human achievement and discovery and discovery which has enabled man to provide his food and fiber needs with progressively less labour.

The first successful domestication of plants by man has recently been suggested to have occurred in Thailand in Neolithic times ..Remnants of rice are broadbeans or soyabeans from fully 10,000 yrs ago were recently discovered.

Harlan lists both the Middle East and Near East as centers and non-centers of agricultural origin.

Much of the record of early agriculture comes from the writings of Greek and Roman scholars such as **Herodotus** about 500 B.C. and **Pliny** about A.D. 50. Hieroglyphs of harvest scenes, and remains of plants and seeds in ancient tombs show an Egyptian agriculture as early as 500-3400 B.C. with the cereal grains emmer and barley of major significance.

Romans of the first century A.D. intertilled crops with iron hand knives. **Woods** writes in 1629 (New England Prospect) in great detail about the skillful use of "clamme shell hooes", used in maize fields to control weeds. Intertillage with animal power was advocated in England in the 17th century.

The value of lime, marl, manures and green manures for the maintenance of soil productivity was recognized 2,000 yrs ago. Books on agriculture written by the Romans (**Pliny**, **Varo** and **Columella**) of about the 1st century A.D. Describe the growing of common crops including wheat, barley, clover and alfalfa by

procedures very similar to those in use today except that more of the work was done by hand and the farm implements then used were crude.

After independence, one of the tasks of the National Government of India was to develop a viable and productive agricultural economy leading to self-sufficiency in our food requirements. Several steps and planned efforts have been made to give effect to this objective. The country is now almost self-sufficient in matter of food grains. With this achievement on India's food-front, the researchers are now being directed for improvement in quality of agricultural produce. However, self-sufficiency in the true sense can be achieved only when each individual in the country is assured of a balanced diet.

Considering the huge economic losses incurred while passing from the field at harvest, to the consumer, "post harvest technology" or "market pathology" is gaining momentum in our country since last few decades after the pioneering work of **Tandon** (1967). During the last few decades, considerable work

in this sphere has been done by many workers. Important contribution have come forth from many countries. Special mention may be made of the work in this field are **Brooks** and **Fisher** (1914), **Horne** and **Horne** (1920), **Baker** (1931), **Brooks** (1933), **Rose** et al. (1939, 1943, 1950, 1951), **Ramsey** et al. (1952), **Smith** (1963), **Smith** et al. (1964), **Simmonds** (1965), **Dainees** (1970), **Wells** and **Harvey** (1970), **Harris** and **Taber** (1970), **Barnett** (1970), **Taylor** (1971) and **Brodrick**, **Westhuizen** and **Van Der** (1976), **Alvarez** et al. (1977), **Mass** (1978), **Seenappa** et al. (1980), **Sommer** (1982) as well as **Eckert** and **Ogawa** (1985).

In India too the problem has received due attention, some important contributions in the field have been made by **Dastur** (1915, 1916), **Mitter** and **Tandon** (1929), **Chona**(1933), **Das Gupta** and **Verma** (1939, 1940), **Singh** (1941), **Sinha** (1946), **Tandon** and **Tandon** (1948), **Verma** and **Kamal** (1951), **Grewal** (1954), **Bhargava** and **Gupta** (1957), **Tandon** and **Bilgrami** (1954), **Tandon** and **Ghosh** (1962), **Ghosh** et al. (1965), **Tandon** (1967, 1970), **Lal** and **Tandon** (1971), **Kapoor** and **Tandon** (1969, 1970), **Dingra** (1970), **Bhargava** (1972), **Raj**

Gopalan and **Wilson** (1972), **Prasad** and **Bilgrami** (1973), **Satyavir** and **Grewal** (1973), **Thind, Saxena** and **Agarwal** (1975), **Dharamvir** (1977), **Vyas** et al. (1978), **Pandey** et al. (1980), **Rai** (1982), **Bharagava** (1982), **Arya** (1982), **Pandey** (1983), **Tewari** (1986), **Srivastava** (1987), **Agarwal** (1987), **Reddy** and **Reddy** (1989), **Mehrotra** and **Pandey** (1989), **Prasad** et al. (1990), **Pundir** et al. (1990) **Malviya** (1992).

The major contribution of plants to human health has always been thought to be the large amount of vitamin A, the folic acid vitamin, and the vitamin C they contained; as well as a good amount of some minerals. It is becoming more and more obvious that there are many plant chemicals that act together to protect the human body from the onset of cancers and heart disease, and that vitamin supplements can be helpful, but are not as useful as the whole plant.

Fruits and vegetables are the only natural resources of protective food as they supply nutrients, vitamins and minerals. In a country where the population is predominantly vegetarian this can only

be achieved by increasing the production and consumption of vegetables.

The importance of fruits and vegetables in human nutrition is well known. Vegetables are rich and comparatively cheaper source of vitamins and minerals. Their consumption in sufficient quantities produces taste, palatability and increases appetite and provides fair amount of fibres. They play key role in protecting against some degenerative diseases. They help in neutralizing the acids produced during digestion of proteinous and fatty foods.

Fruits are undoubtedly an integral part of human's food. They are prized as a source of refreshment, for their delightful flavour and aroma. Their history is perhaps as old as that of Adam, Eve and the forbidden apple. The records dating back to 7,000 B.C. indicate that date palm was perhaps the earliest fruit cultivated by man. Pomegranate was grown as early as 3,500 B.C. References to peach and almond are found in Egyptian manuscripts written around 1300 B.C. Mention of several fruit like amla,

bael, wild dates, figs, grapes, karonda, lemon, mango, mulberry, orange, phalsa, banana and pomegranate are made in early medical works in Sanskrit, the Charaka Samhita and the Sushruta Samhita, Vriksha Ayurveda, written in 1392, deals with fruit culture and propagation, including graftage.

Today, fruits are absolutely essential for the maintenance of health. They are rich in carbohydrates, minerals and vitamins, and are an integral part of the balanced diet of man. They are an important source of both digestible and indigestible carbohydrates.

The strawberry is very rich in potassium, natural, sugars, vitamins and mineral elements. It has about 80-90 percent of water content, which helps in cleansing the entire system. Likewise, Peach is also rich in potassium; calcium and sodium. Pomegranate has 77 percent water content and is extremely rich in sodium, vitamin A, B and C. It contains glucose, fructose, tannin and oxalic acid. The fig is another fruit of importance. It is a rich source of sugar, protein, fat and carbohydrate. The peel of the apple

is extremely rich in vitamin A, whereas the actual fruit contains vitamin C, B-1, B-2, B-6, folic and pantothenic acid. Minerals such as potassium and small amounts of copper, magnesium and phosphorous are also present is apples. Pineapple has great nutritive value. It contains carbohydrates, proteins, fats and water. It also has calcium, phosphorous, iron, magnesium, potassium and sodium, chlorine, sulphur and manganese. These are an excellent source of vitamin C and has vitamin A, B1 and B2. Avocado is high in vitamin E, monosaturated fats and calories. It has a good quantity of calcium, iron and phosphorous as well as almost all vitamins. Mangoes are rich in vitamins A and C. They also have iron and beta-Carotene. Grapes are incredibly rich in vitamins A, C and P(bioflavonoids) and trace elements such as germanium and selenium . Raisins and sultanas are a concentrated sources of colories, sugar, and nutrients. Papaya is a valuable fruit having vitamins A, B, C and D. Banana is a good source of potassium, sodium, phosphorous, chlorine, magnesium, sulphur, silicon and calcium. They are rich in vitamins A, B1,

B2, B5, and C. Lemons are very rich in citric acid, and vitamin C. They also have phosphorus, magnesium, potassium, sodium and calcium. Fresh grapefruit is a good source of flavonoids, water-soluble fibres, potassium, vitamin C and folic acid. Melons are a good source of potassium and vitamin C. Oranges have vitamin C and flavanoids. They provide protein. Prunes are rich in magnesium, sodium, phosphorus and potassium. Tangerine had high content of phosphorus and calcium and is also rich in magnesium and vitamins. Tomatoes have substantial levels of antioxidants, vitamin E. Water melons are valuable for their minerals, vitamin, sugar and pure water. Dates are high in potassium and dietary fiber and also a good source of iron. The skin of pear is a good source of dietary fiber and have a high content of sugars, minerals and vitamins. Plums have a high content of magnesium, sodium, phosphorous and potassium. Guavas are excellent source of Vitamin-C.

Nutrients are usually divided into five classees - Carbohydrates, Proteins, Fats (including oil), Vitamins and Minerals. We also need fiber and

water. All are equally important to our well being, although they are needed in varying quantities, from about 250 gm of carbohydrates a day to less than two micrograms of vitamin B12. It is necessary to balance the complementary amino acids in a vegetarian diet.

Carbohydrates are our main and most important source of energy, and most of them are provided by plant foods. There are three main types : simple sugars, complex carbohydrates or starches and dietary fiber. The sugars or simple carbohydrates can be found in fruit and ordinary table sugar. Complex carbohydrates are found in cereals/grains (bread, rice, pasta, oats, barely, millet, buckwheat, rye) and some root vegetables, such as potatoes and parsnips. The unrefined carbohydrates, like wholemeal bread and brown rice are best of all because they contain essential dietary fiber and B vitamins. Fiber can be found in wholegrain cereals, fruit (fresh and dried) and vegetables.

Too much fat is bad for us, but a little is necessary to keep our tissues in good repair for the

manufacture of hormones and to act as a carrier for some vitamins. Two of these fatty acids, linoleic and linolenic acids, are termed essential as they must be provided in the diet. This is no problem as they are widely found in plant foods.

Vitamin is the name for several unrelated nutrients that the body cannot synthesize either at all, or in sufficient quantities. Many vegetables contain a substance known as carotene which is converted into Vitamin A in the body. Generally, deep green yellow and orange coloured vegetables, such as green leafy vegetables, carrots, papaya, tomatoes and yellow pumpkin are rich sources of carotene . Fruits like apricots and peaches are also good source of vitamin A. B group of vitamins includes B1 (Thiamin), B2 (Riboflavin), B3 (Niacin), B6 (Pyridoxine), B12 (Cyanocobalmin), folate, folic acid, pantothenic acid and biotin. All the B Vitamins except B12 occur in yeasts and whole cereals, nuts and seeds, pulses and green vegetables like fenugreek leaves, turnip greens and beet green. Vitamin B12 is the only one that is not present in plant, foods. Only little amount of B12

is needed which is added to yeast extracts, soya milks, veggie burgers and some breakfast cereals.

Vitamin C is contained in good amount in fresh fruits, salad vegetables, potatoes, all leafy green vegetables, Indian gooseberry, bitter gourd, tomatoes, spinach, cabbage and drumstick leaves. Vitamin E is present in vegetable oil, whole grain cereals, leafy greens. It is needed for growth, muscle tissues, normal reproduction. Vitamin K is present in green leafy vegetables, soyabean oil and cereals.

The highly soluble minerals like calcium, iron, phosphorus, magnesium, copper and potassium contained in the vegetables maintain the acid-base balance of the hydrogen concentration of the body tissues. Calcium can be found in : Watercress, rhubarb, beets, parsley, spinach, broccoli, chinese cabbage, raw onions, raw celery, okra, chives, raw cabbage, cucumbers, turnips, zucchini, green beans, squash, artichokes, leafy green vegetables. Iron is essential for proper formation of red blood cells and regulation of body processes. Iron can be found in plenty from

leafy vegetables like spinach and fenugreek leaves, in prunes, raisins, nuts, dried fruits (especially apricots and figs). Manganese cán be found in legumes, nuts, fruits. Zinc can be found in nuts and seeds, yellow and green veggies, yellow fruits, pumpkin, sesame seeds, etc.

Proteins can be obtained from various sources like nuts, pulses, cereals and vegetables like pumpkin, peas, beans, etc.

Vegetable contain various medicinal and therapeutic agents. There are a large array of laxatives, sedatives and sleep inducing in the vegetable kingdom. Certain vegetables are highly beneficial in the treatment of various diseases. Carrots are good for the blood. White crisp juicy stalks of celery serve a much better medicine in case of rheumatism or nervous dyspepsia than any nervine that relieves nerve disorder. A dish of spinach or dandelion will be beneficial in the treatment of kidney troubles. Lettuce can be used as a food remedy for insomnia. Onion can be used with advantage in the

treatment of cough, cold, influenza, constipation, scurvy and hydrophobia. The leaves of fenugreek are highly valuable in the treatment of indigestion, flatulence and sluggish liver. Garlic can be beneficially used in heart diseases, hypertension, hypoglycemia, diabetes and even in fatal form of meningitis. It has been effectively used in lowering blood cholesterol and preventing blood clotting. Beet root, cabbage, carrots, cucumbers, green peas and beans are especially valuable in this. They are useful in case of arteriosclerosis, high blood pressure and constipation. Pectin found in vegetables such as brinjal, radish, pumpkin and beet root absorb water, kill certain bacteria and toxins and eliminate them from the body.

Carrots are a super food source of beta carotene, a powerful anti cancer, artery protecting, immune boosting, infection fighting antioxidant with wide protective powers. Parsley is an anticancer herb due to the presence of high concentrations of antioxidants such as monoterpenes, phthalides, poly acetylenes. Beets are good for lymph nodes and the

heart. Aids in fighting cancer. Reduces fat. Helps lower blood pressure, prevent osteoporosis helps blood to clot. Broccoli is a powerful antioxidant. Removes toxins. Cabbage juice helps heal ulcers. Spinach and other green leafy vegetables have Lutein, an anti-cancer compound. Tomatoes are a major source of lycopene, a strong anti-oxidant and anti-cancer agent. Garlic lowers cholesterol, blood pressure., and is an anti-viral and anti-bacterial food.

Similarly fruits have natural powers of curing numerous diseases, aches and pains. Apples have mild anti-bacterial, anti-viral, anti-inflammatory and anti-cancerous activity. They lowers the risk of heart disease. Oranges contain a complete package of every class of cancer inhibitor known-carotenoids, terpenes and flavonoids.

Pineapples contain bromelain which aids digestion, suppresses inflammation, is good for the joints and muscles and reduces swelling. It helps dissolve blood clots & is good for preventing osteoporosis and bone fractures because of its high

magnesium content. The medicinal value of fingered citron is very great. It helps in digestion, relieve cough and reduce sputum, smooth the liver, and strengthen the spleen. Its fruits can cure the tummy bug, vomits, choke, high blood-pressure, tracheitis, asthma and so on.

Dried figs are well known for their laxative effect. Grapes benefit the blood system. They purify and enrich it with red globules. Strawberries are beneficial for the intestinal tract, liver, kidneys and the heart. It is supposed to cure rheumatic disorders. Externally, it can be used to heal old wounds, sore eyes and ulcers. Eating peach along with its skin can cure chronic constipation. It is a good natural cleanser for the kidney and bladder. The pomegranate is an excellent heart tonic. It increases resistance towards tuberculosis and tones up the heart, liver and kidneys. The dry powder made from its buds is used to cure diarrhoea as well as nose bleeds. The juice of the fruit is excellent for digestive disorders. Leaves can be used to heal wounds and cuts. Figs are extremely beneficial for curing piles,

phlegmatic cough and urinary diseases. It has been used to treat boils from time immemorial.

Stakman and **Harrar** (1957) defined plant disease as, ***"a physiological disorder or structural abnormality that is harmful to the plant or to any of its part or products that reduces the economic value".***

Man is directly dependent upon plants for his survival because plants are his prime source of food, fibre and drugs. Plants are also important to man because they utilize Co_2 in photosynthesis and release O_2. Phytopathology is the study of the diseases of plants and covers the entire field of biological and scientific activity concerned with the understanding of this complex phenomenon. Phytopathology is thus the study of the nature, development and control of plant diseases.

Disease, a complex phenomenon, is difficult to define in a few words. A simple dictionary meaning of disease is, "any departure from health, presenting marked symptoms, malady, illness, disorder". A plant disease is broadly defined as any condition in which a

plant differs in some way from a normal plant in either structure or function. The plant may be shorter, have more branches or fewer leaves than normal or differs in structure. Or, it may will and die prematurely, or not produce flowers or fruit, its function differs. Diseased plants are distinguished by changes in their morphological structures or physiological processes which are brought about by unfavorable environment or by parasitic agencies. According to the modern conception, disease is an interaction among the host, parasite and environment.

People have been aware of plant diseases since the dawn of civilization. Ancient texts often described illness manifested in plant to some kind of divine retribution (Holy Bible, read Deuteronomy Vrs 12:11-16). In fact, it was not until the latter half of the 10th century that, paralleling medicine, microorganisms were proven to be the cause of many plant diseases.

Plant diseases have had an important role in history. The holy fire in the Bible is believed to

have been due to the ergot fungus in the heads of grain which made bread poisonous. Many of the plant diseases, such as chestnut blight, coffee rust, ergot of rye, potato late blight and rust and smuts of cereals have had a major influence on world history leading to wars, famines, mass poisoning and mass emigration.

Irish famine (1946-1950) is an example of the tremendous impact that plant disease can have on the course of human history. In the period between 1846-1850, the late blight of potatoes, caused by the fungus <u>Phytophthora infestans</u>, resulted in a famine among the working class population of Ireland. So sudden and so complete was the catastrophe that in only a few days, fields which had promised abundant harvests, were transformed into blackened waste of vegetation overlying foul and putrefying masses of rotten tubers. As harvest across Europe failed the price of food soared. Peasants who ate the rotten produce sickened and entire villages were consumed with cholera and typhus. The Irish famine took as many as 1 million lives from hunger and disease and changed

the social and cultural structure of Ireland in profound ways. The combined forces of famine, disease, and emigration depopulated the Island.

In the early 1870s another fungus disease, the **coffee rust** caused by Hemileia vastatri, wiped out the coffee plantations of Sri Lanka. Coffee growing was, therefore, abandoned, at least for many decades, and much of the world's coffee production was shifted to the western hemisphere where the disease does not occur. The tea and rubber industry replaced coffee in Sri Lanka and other eastern areas.

Chestnut blight caused by the fungus Endothia parasitica nearly wiped out American chestnut. The first report of the disease came from the New York Zoological Park in 1904. Although the causative agent of the disease was soon discovered, no suitable control measures could be found. The result was that about 30 billion board feet of wood, used for furniture, flooring, poles, etc. was lost. The loss of one species created far reaching effects that have not been completely overcome till now.

In India, the 1942 **Bengal famine** was perhaps largely due to the Helmithosporium disease of rice, caused by Helminthosporium oryzae Breda do hann. About two million people died of starvation. According to **Padmanabhan**, (1973), nothing as devastating as the Bengal epiphytotic of 1942 has been recorded in plant pathological literature.

Another instance of a serious loss by a fungus disease is due to the red rot of sugarcane caused by Physalospora tucumanensis. It reached its peak in 1938-39 in the white sugar belt of northern India - Bihar, Punjab and Uttar Pradesh. In the badly affected areas, most of the mills could crush only 33 percent of their normal quantity (**Woodhead** et. al. 1945).

The previously mentioned few examples amply prove that diseases can entirely change the course of history and the economy of a country, and they have been and still are a limiting factor in crop production.

Information on internationally important plant diseases exists (**Holliday**, 1971; **Klin kowski**, 1970;

Meredith, 1973; **Padwick**, 1956; **Riker**, 1964). Some of the plant diseases which can assume serious proportions have been ably discussed by **Thurston** (1973). Some common epidemics have been recently discussed by **Horsfall** and **Cowling** (1978).

The advent and progress of human civilisation is intimately associated with the progress of agriculture. But ever since man started depending on plants to fulfil his nutritional needs, the enemies of plants have been causing much harm and economic losses. Fossil records of parasitic fungi date back to Devonian period which suggests that plant diseases originated along with the plants. In India, where agriculture is nearly 4,000 years old, mention of plant disease has been made in Rigveda, Atharveda (1500-500 B.C.), the Artha Shastra (321-186 B.C.), Sushruta Sanhita (200-500 A.D.), Vishnu Puran, (500 A.D.), Agnipuran (500-700 A.D.) etc. In Europe, **Theophrastus**, while describing trees, cereals and pulses also recorded the harmful effects of wind, weather and location and believed that diseases originated from plants or from the environment. But

the invention of the microscope changed the entire concept **Micheli** in 1729 made an extensive study of fungi and their reproductive structures and experimentally proved that the fungi originated from spores. In 1755, **Tillet** demonstrated that wheat seeds dusted with bunt disease of wheat was caused by transmission of black powder (bunt) and was contagious.

The foundation of modern plant pathology as a science was established by the French scientist **Prevost** (1807) who demonstrated that micro-organisms are disease causing. An important discovery made by **Prevost** was the fungicidal and fungistatic properties of certain chemicals. But due to the firm believe of scientists in the theory of spontaneous generation, **Prevost's** descoveries were acknowledged 40 years later. The German Scientist **Anton de Bary** is credited for the establishment of modern experimental plant pathology. **De Bary's** outstanding contributions are his studies with rusts, smuts, downy mildew fungi, hetroecious natures of rusts and their pathogenic role. The role of enzymes in tissue disintegration was

demonstrated by **De Bary** and the physiological era in plant pathology was initiated.

The role of fungi in disease development and the observations of the various scientists was recorded by **Kühn** in 1858 in the first book of plant pathology. A big step forward in the field of plant pathology was made by **Brefeld** of Germany (1875) who developed the modern techniques of inoculation and the artificial culture of micro-organisms. Parasitic fungi were now being cultured in sterile synthetic, media to study their physiology, nutritional requirements, sporulation and genetics. The variability of parasitism in pathogens and the existence of physiological races in the rust fungus was discovered by Swedish scientist **Erikson** in 1984. In the first decade of the 20th century, scientists studied the importance of genetics in plant disease resistance and selection of resistant lines was introduced to obtain resistant varieties (**Biffer**, 1903 and **Orton**, 1909). At the same time. **Ward** (1903) and **Salmon** (1903) discovered physiologic specialisation in rust and powdery mildew of cereals. **Hansen** and **Smith**

established the theory of origin of physiologic races through heterokaryosis.

The extremely large number of plant diseases makes it impossible for any person to be familiar with all of them. However, there are certain facts that help in the identification of plant diseases. The most important is to know the name of the plant that is affected. Then one can check known plant diseases to see if a similar condition has ever been reported on that plant. How the disease developed is also important. If it occurred in a very short time (Overnight), it is probably not a parasitic disease, but is more likely due to some unfavourable environmental condition or chemicals. Parasitic disease usually do not affect a large percentage of the plants in the early stages, but start in one area and gradually spread to the other plants. And, parasitic diseases usually do not affect several different kinds of plants in one area at one time, even though some disease organisms can attack many different plants.

For so far as it is possible to see into the future, mankind will depend, as it always has, on the plant world for food and fiber and a range of other essential materials.

Whatever else may be the implications of plant disease, its most direct impact is to reduce the quantity or quality of the materials derived from the agricultural lands, the ranges and the forests.

Plants, at least the majority of the common crop plants, may for convenience be thought of as composed of a stem-root axis, the stem bearing leaves and flowers. By examination of the basic structure of these parts and their functions we can gain some insight into their impairment by disease.

Taken as a unit, it is the root system of the plant through which virtually all of the water and dissolved substances enter, and it is this system which anchor the plant to its site. To varying degrees food materials are stored in root tissues, and not a few plants have strong tendencies toward vegetative reproduction of these parts. Diseases may materially

reduce the absorptive capacity of the root system, as in the case of the root rot fungus, bacterial crown gall, clubroot of crucifers, and the ubiquitous nematode root knot. Impairment of the supporting properties of the roots is most apparent in such crops as corn, where the fungus root rots are responsible for premature collapse of a large number of plants. Once on the ground, ears are very vulnerable to rot fungi, with consequent reduction in total marketable field. The Rhizopus soft rot of the sweet potato in storage is an example of damage to the storage function of that root.

The aerial portion of the plant axis serves chiefly to maintain the leaves and flowers in position favourable to their particular "needs". As such, the stem is a supporting and conducting organ, secondarily of importance as a place of storage, and not infrequently a means of vegetative reproduction. Whereas the roots of plants are useful to man as sources of food, of medicines and of dyes, and indirectly as a soil retaining element, the stems are the source of timber and timber products, fiber,

natural gums, resins and latex. There are a considerable number of wilt diseases which adversely affect conduction in stems. The Fusarium wilts of watermelon, cotton and other crops, the bacterial wilt of corn, cucumber and other cucurbits will serve as examples. Some controversy yet remains over the exact mechanism of this damage, but it is probably a mechanical plugging of the Xylem elements, perhaps augmented by toxin activity. Among diseases which reduce the supporting properties of stems, the Diplodia stalk rots of corn, the numerous stem rusts of small grains, and perhaps above all the heart rots of timber and fruit trees may be cited.

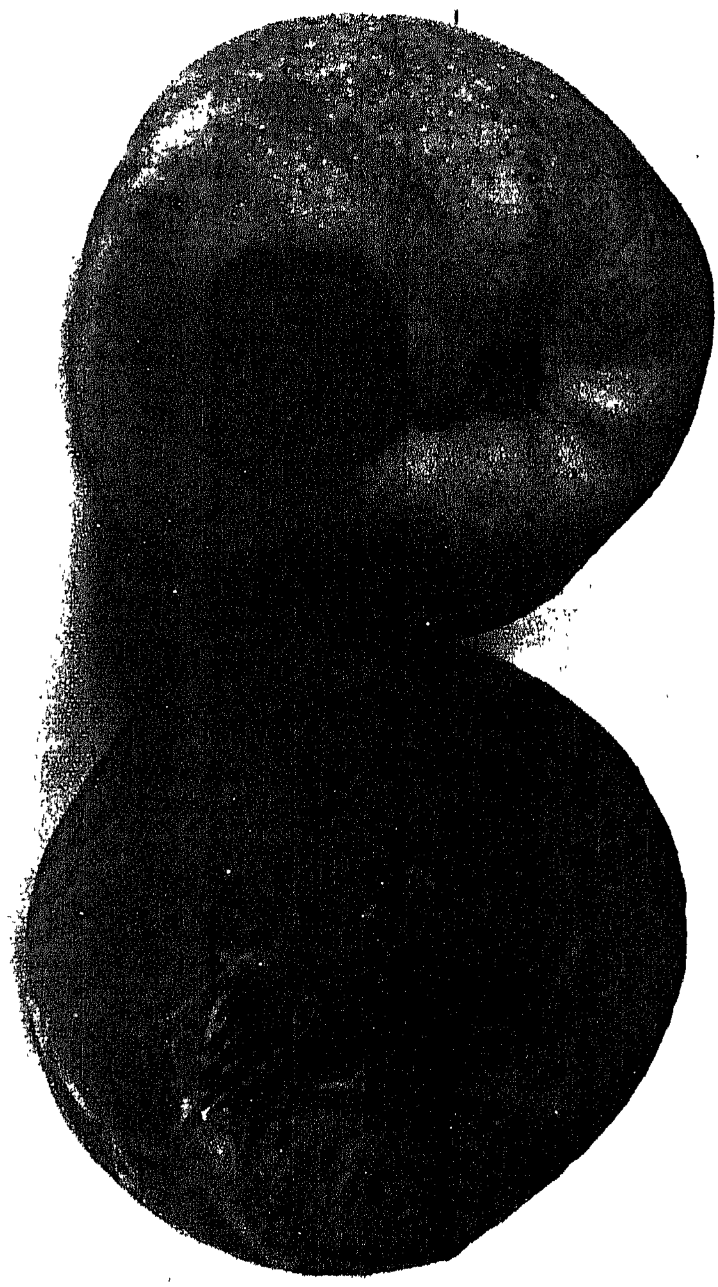
As varied as the leaf may be, it has one major role, that of photosynthesis. Whatever may be the implications thereof, the unmodified lead is admirably constructed for this purpose. By all odds the greatest value of leaves to man is indirectly through the products of photosynthesis, although they serve also as the principle source of the "protective" foods, i.e. vitamins, and of certain drugs and beverages. It is, therefore, those diseases which decrease the

photosynthetic activity of the leaf with which we are most concerned. This effect may result from a masking of the leaf surfaces, as in the case of the sooty molds of citrus and other plants, or by the destruction of restricted areas in the leaf, in the case of the apple black rot "frogeye", the tar spot of maple, and the leaf rusts and "shothole" diseases. Many of the virus diseases reduce the size of the leaf, hence the amount of photosynthetic tissue. Many reduce the amount of chlorophyll in certain areas, producing the widely recognized mosaic effect. If, as in the apple scabs infection, the petioles are so injured as to induce leaf drop, a corresponding reduction in food manufacture results.

As the leaf has but one major "function", so too does the flower, that of the production of fruits and seeds, Ultimately, all higher plants exist by virtue of the fact that they produce fruits and seeds. This process involves the combination of germ plasms in a process known as fertilization, and perhaps because of this the essential parts of flowers are regarded as being limited to the stamens and the

pistil. Sepals, petals, etc., are considered auxiliary. Except perhaps as ornament, flowers are of importance to us primarily as precursors of fruits and seeds, which in turn furnish most of our food supply. The list of diseases attacking the flowers and fruits of our commercial crops is seemingly endless. Examples include the blossom infections of the peach by brown rot fungus, the bacterial blight of pear and apple (fire blight, and some of the smut fungi. A consideration of the diseases of the seeds and fruits would include the smuts of most small grains, the numerous corn ear rot fungi, the nematode infections of wheat, the fungus and bacterial rots of grain and fruits in storage.

MATERIALS AND METHODS



Scientific methods are not scientific unless they are practical, and they are not practical unless they are scientific.

[H.H. Whetzel]

Most phases of plant pathology are of vital concern to consumers. Inevitably, consumers as a group bear practically all the cost of losses from plant diseases and derive the larger portion of the benefit of disease control. 'Market pathology' as a somewhat specialized field is of particular interest to a special group of consumers; namely those who live a significance distance from the areas where their food is produced. The more remote either in time or space the area of consumption of fresh fruit and vegetables is from the area of production, the more importance are diseases which cause losses between producer and consumer. Diseases occurring on fresh fruits and vegetables while in transit, in storage, and on the market and the means of controlling those diseases constitute the special field of market pathology. The

various fungal organisms are responsible for a large number of diseases of plants.

It, is, therefore, necessary to survey the post harvest diseases of fruits and vegetables in and around Allahabad district. A broad survey of Allahabad was undertaken to collect the samples of infected fruits and vegetables. A large number of infected fruits and vegetables were observed. They were collected in sterilized polythene bags before bringing to the laboratory for their study.

In the laboratory, the infected areas of vegetables and fruits were observed carefully and the symptoms were recorded. After that usual methods of isolation and purification were adopted. The surface area of the infected fruits and vegetables were sterilized using 90% alcohol. The isolations were done in the doubledoor inoculation chamber. Few pieces of the fruits and vegetables were cut from the junction of healthy and infected parts, using the sterilized razor. These pieces were then transferred aseptically to petridishes containing Potato Dextrose Agar Medium

(200gm. potatoes; 20gm., agar-agar; 20gm. dextrose; 1000cc. water).

The cultures were made bacteria free by the methods suggested by **Brown** (1924). The fungal colonies developed after sometime were examined and identified. The pathogenecity of the causal organisms was established only when **Koch's** (1882) postulates were fully satisfied. **Koch's** postulates can be briefly summarized as follows -

1. A specific organism must always be associated with a disease.

2. The organism has to be isolated in pure culture.

3. The organism must be identified.

4. The organism must produce disease in a healthy susceptible host.

5. The organism must be isolated from the experimental host, again in pure culture, and its identity established.

During the investigation large number of pathogens were isolated from different hosts. However, out of so many pathogens only 3 fungi namely -

(1) Alternaria alternata from Pineapple,

(2) Fusarium oxysporum from Snake gourd, and

(3) Botryodiplodia theobromae from Carambola

were finalized for the further detailed pathological studies. Single pore cultures of these pathogens were prepared by using the dummy cutter objective as described by **Keyworth** (1959). The pathogenecity of the causal organisms were established by **Koch's** postulates and they were found to be pathogenic to their respective hosts. Stock cultures were maintained on PDA medium.

Only Pyrex or Corning brand glasswares, B.D.H. and E. Merch brand of chemicals of analytical grade were used. The glasswares were always cleaned with dilute chromic acid solution. ($K_2Cr_2O_7$ - 100 g, Conc. H_2SO_4 - 100 ml and distilled water 600 ml.) After that

they were thoroughly washed with tap and finally with distilled water.

The pathogenecity of the disease causing organisms under investigation was tested by the following three different methods :-

(1) **Granger and Horne's (1924) method** :

In this method, a sterilized cork-borer was inserted in the healthy fruit and then carefully pulled out with a little flesh of the fruit. Now the inoculum consisting of mycelium and spores of the organism was then placed in the bore. Now the piece of the flesh previously removed was inserted back to its original place. The cut surface was then sealed with the help of wax. The control was treated in the same way but no inoculum was placed in the pit.

(2) **Injury Method** :

In this method, the fruits were injured with the help of a sterilized needle and the

inoculum was placed over the injured area. Controls were treated in the same manner except that no inoculum was placed over the injured regions.

(3) **<u>Spore Suspension Method</u>** :

In this methods, the spore suspension containing uniform number of spores of respective pathogen was prepared in sterilized distilled water. It was then sprayed with the help of an atomizer on the injured as well as uninjured surface of respective host fruits. Fruits sprayed with sterilized distilled water containing no spore suspension served as control.

The inoculated fruits were always kept separately in sterilized polythene bags to prevent any secondary infection. Cross-inoculations were also carried out on a large number of fruits to investigate the possible host range of the pathogens. Re-isolations were invariably made in order to confirm the infection with particular organism.

Hanging drop technique as described by **Hoffman** (1860), was used to study the mode of spore germination as well as the effect of various factors viz., nutrients, temperature and pH on the germination of spores. For this purpose spores were taken from 15 days old cultures.

The thermal death point of the organisms was determined by usual methods. Spore suspension of each pathogen prepared in sterilized distilled water was filled in fine capillary tubes. Subsequently, both the ends of the capillary tubes were sealed. This sealed capillary tube containing the spore suspension of the organism was placed at various prefixed temperatures for 10 minutes. These capillary tubes were then crushed in petridishes containing solid basal medium. The absence of their growth indicated their death point at that particular temperature.

Alcoholic extract of the healthy and diseased fruit tissues were analysed chromatographically to study the post infection changes in amino acid, organic acid and sugar contents

during pathogenesis. 2 gm of the fruit tissues of healthy as well as infected fruit was taken out and was thoroughly crushed in a ground glass homogenizer with 25 ml. of 80% ethanol. The crushed substance was boiled over an electric water bath for 30 minutes and subsequently filtered through Whatman filter paper No. 1 (circles). The residue was washed three or four times with 10 ml. of hot ethanol. The filtrate was then evaporated to dryness and this is dissolved in 2ml. of 20% ethanol. The solution was centrifuged for 30 minutes at 2000 r.p.m. The clear supernatant liquid in the centrifuge tube was decanted and this alcoholic extract contained free amino acids, organic acids and sugars.

To release the amino acids from the proteins, the alcohol extracted residue on the filter paper containing protein fraction and the colloidal protein from the centrifuge tube were combined and hydrolysed with the help of 6NHCl in an autoclave at 15 lbs. pressure for half an hour. A pinch of stanous chloride was added to avoid humin formation by destruction of amino acids in presence of

carbohydrates. The hydrolysed residue was filtered through a Buchner funnel, using Whatman filter paper No.1. and the hydrolysate obtained was so adjusted as to contain 1ml per gram of fruit tissue. After centrifugation, the supernatant was used for chromatographic analysis of protein bound amino acids.

Two dimensional ascending paper chromatographic technique as described by **Consdon** et al. (1944) was used for the detection of amino acids. Solvents used were phenol : ammonia : water (80:3:20, v/v) in first direction and n-butanol: acetic acid: water (4:1:5, v/v) in the second direction (right angle to the first) 0.1% ninhydrin in n-butanol (w/v) was sprayed to locate the position of amino acids. Chromatograms were developed at 80^0C for 20 minutes.

Proline was not detected by the method outlined above. So, for this purpose, one dimensional ascending paper chromatographic technique was used. The solvent was n-butanol:acetic acid:water (4:1:5,v/v). Proline, which gives a weak yellow spot with ninhydrin, was detected by spraying isatin

reagent of **Saifer** and **Oreskes** (1954). It contained 0.2% (w/v) of isatin in acetone and 4% (v/v) of acetic acid. It was sprayed on dry chromatograms. After drying at room temperature the chromatograms were heated at 100^{0}C for 10 minutes. Protein appeared on the chromatogram as a navy-blue spot.

The identity of various amino acids was confirmed by co-chromatography of known amino acids and the intensities of the spots were compared visually and, according to their concentration, graded into four categories, +, 2+, 3+ and 4+. The sign (-) denoted the absence of amino acid.

For chromatographic analysis of organic acids, one dimensional ascending paper chromatography as described by **Lugg** and **Overall** (1947) was employed. n-butanol: formic acid: water (10: 2: 5, v/v) were used as running solvent. Chromatograms were sprayed with 0.04% bromophenol blue (w/v) in ethanol. Organic acids appeared as lemon yellow spots. For quantitative estimation of organic acids, the area of different spots was measured separately with the help of a

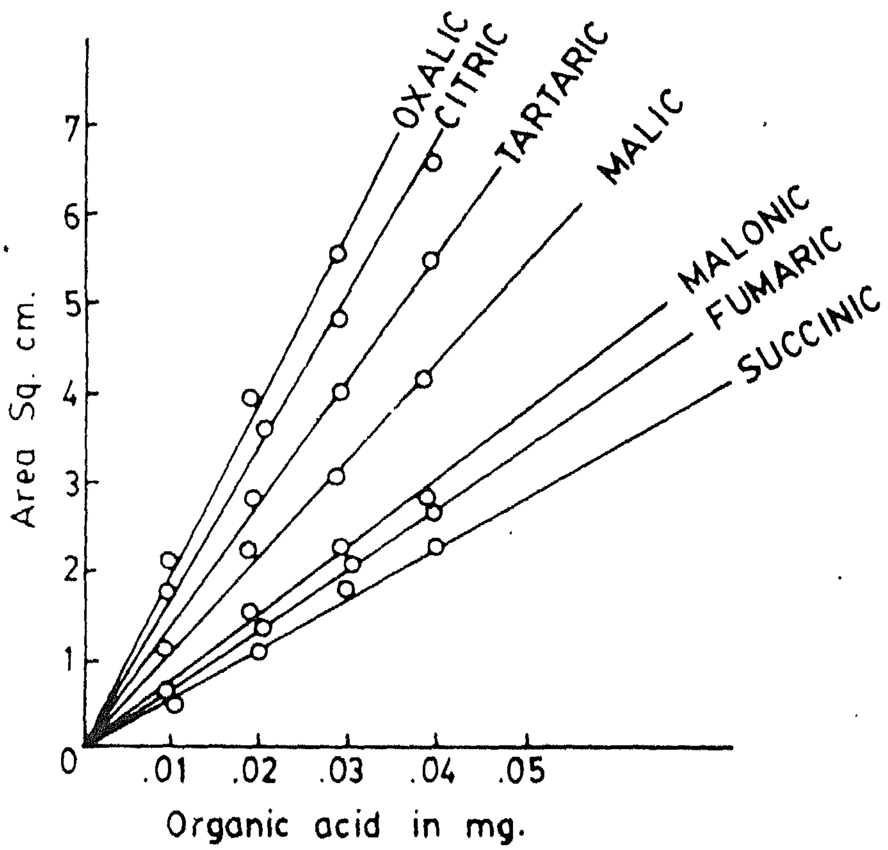
planimeter and the values thus obtained were expressed quantitatively in terms of area occupied by known amount of respective organic acid from predetermined calibration curves (Text Fig.1)

The presence of different sugars in healthy and diseased fruits were detected by one dimensional ascending paper chromatographic technique. Rest of the procedure was similar to the one described earlier. The intensity of spots was observed visually.

The identification of various organic acids and sugars on the chromatograms was confirmed by the co-chromatography of the standard known substance.

Estimation of Vitamin C (ascorbic acid) of diseased and healthy fruits was made quantitatively. It was determined by titrating the extracts against a standardized solution of 2,6-dichlorophenol indophenol (**Bessey** and **King**, 1933). The solution of this reagent was prepared by dissolving 25 mg of it in 100 ml of distilled water. It was then warmed a little and filtered. A pinch of sodium bicarbonate was added to prevent oxidation. Standard ascorbic acid solution was

PREDETERMINED CALIBRATION CURVES OF ORGANIC ACIDS



TEXT FIG. 1

prepared by dissolving 5mg of pure L-ascorbic acid in 100ml of 5% (w/v) metaphosphoric acid. Phenol indophenol solution was always standardized before using it against fruit extract.

Extracts for analysis of ascorbic acid were prepared in the following manner. 2g of healthy and diseased fruit tissue were separately crushed in 25ml of 5% metaphosphoric acid and filtered. The residue was washed twice with 10ml of metaphosphoric acid and volume was finally raised to 50ml by adding required amount of metaphosphoric acid. 10ml. of filtrate was titrated against previously standardized solution of 2,6 - dichlorophenol indophenol. The volume of indophenol reagent required for completion of each titration was recorded on the basis of three readings. In all cases blank correction of the titration value was made as suggested by **Franke** (1955). It was first done by titrating the indophenol reagent against 10 ml. of 5% metaphosphoric acid solution required to impart perceptible pink colouration. This volume was deducted from all subsequent titration readings. The

quantity of ascorbic acid in mg/100gm fruit tissue was calculated from the following formula -

$$\frac{A \times I \times V \times 100}{v \times W}$$

Where,

A = Quantity of ascorbic acid (in mg) reacting with 1ml of indophenol.

1	=	Volume of indophenol solution required for the completion of titration with extract.
V	=	Total volume of extract (here, 50 ml).
W	=	Weight of fruit pulp (here, 2g).
v	=	Volume of the extract used in each titration (here, 10ml).

Poisoned food technique as described by **Nene** and **Thepliyal** (1979, pp. 413-414) was used for the evaluation of different fungicides and antibiotics at various concentrations in the laboratory. The particular concentrations of different fungicides and antibiotics which were found effective against pathogens <u>in vitro</u> have been tried <u>in vivo</u>.

ISOLATION STUDIES

Plant Pathologists are squirt gun botanists.

[R. Thaxter, 1891]

Earlier the food value, diseases, and importance of vegetables and fruits have been discussed in great detail.

Before describing the fungi isolated during the survey, it is necessary to know the structure and economic importance of fungi.

The study of fungi was diligently pursued by several able systematists like **Bulliard, Batsch, Persoon, Link, Schweinitz** and **Fries.**

In the first 75 years of the 19th century, the microscopic study of all sorts of fungi, e.g., rusts, smuts, various moniliales and pycnidial fungi helped in their proper identification.

De Bary (**Heinrich Anton De Bary,** 1931-88) is called the founder of modern mycology. His mycological

interests were more biological than taxonomic. His investigations ranged from study of life histories to elucidation of the subtle mechanisms of parasitism and saprobism (saprophytism) to the nature of lichens etc.

Oscar Brefeld (1839-1925) studied fungi by growing them in pure culture under varying conditions.

De Bary, after proving that the 'late blight' disease of plants was caused by the activity of a fungus, Phytophthora infestans, ushered in the era of "Physiological Plant pathology" by suggesting the involvement of toxins and enzymes in plant diseases. Some of those whose studies have a historical significance are: the studies of **Van Tiegham** (1867, conversion of tannin to gallic acid by fungi); **Raulin** (1867, Mineral nutrition of fungi); and **Wehmer** (1891, organic acid production from sugar by fungi).

Fungi compromise a separate major group of organisms which differ from plants in origin, direction of evolution and organization due to adaptation to different mode of primary nutrition. They are heterotrophs dependent on external supply of

readymade organic food, which they produce as parasites or saprobes. Unlike animals, which are also heterotrophs, their nutrition is absorptive, ingestion is rare and restricted only to the slim molds. The hyphae lie in direct contact with the nutrients and absorb dissolved smaller molecules such as simple sugars and amino acids. Large insoluble substances like polysaccharides, fats, proteins, etc. are first broken into smaller fragments until the soluble monomers are released. This is called digestion and is achieved by extra-cellular enzymes.

Fungi are too diverse to be easily defined. The main characteristics are the following (Adopted from **Ainsworth**, 1973). Nutrition is heterotrophic (Photosynthesis lacking) and absorptive (ingestion rare). Thallus on or in the substratum and plasmodial, amoeboid or pseudoplasmodial; or in the substratum and unicellular or filamentous (mycelial), the last septate or non-septate, typically non-motile (with protoplasmic flow through the mycelium) but motile states (e.g., zoospores) may occur.

Cell Wall is well defined, typically chitinized (cellulose in Oomycetes). Nucleus is eukaryotic, multinucleate, the mycelium being homo-or-hererokaryotic, haploid, dikaryotic, or diploid, the last being usually of limited duration. Life cycle simple to complex. Asexual or sexual reproduction and homo-or heterothallic. Microscopic or macroscopic sporocarps and showing limited tissue differentiation. Ubiquitous as saprobes, symbionts, parasites or hyperparasites and cosmopolitian.

Fungi exhibit both harmful and useful activities to Human beings. Some of them at times cause serious diseases while others are of immense importance to green plant and mankind.

The food value of fungi is well-known since remote past. About 2,000 species of mushrooms are known to be edible for instance Agaricus compestris, A. bisporus, etc. Yeasts contain a complete protein consisting of large number of free amino acids besides fats, mineral salts, vitamins, etc. Fungi are widely used in industries such as baking, brewing and cheese

industries and also in the production of organic acids, solvents, vitamins, etc. Fungi have explored a new field in medicine by producing antibiotics and several other useful components like ergotin and ephedrine. Fungi maintain soil fertility by decomposing the dead organic matter and also prevent the loss of inorganic material by leaching from the soil. Neurospora, Saccharomyces, Sordaria, etc. are frequently used for the purpose of genetical and biochemical studies. Certain fungi are used for controlling soil borne pathogens. The well known growth promoting substance Geibberellic acid is obtained from Gibberella fujikuroi.

Besides being useful to mankind, fungi causes great losses to the mankind. Fungi belonging to Mucorales, yeasts, Aspergillaes, Moniliales, etc. are largely responsibly for food spoilage in nature. Rhizopus nigricans, Penicillium digitatum, Zygosaccharomyces, Debromyces, etc. spoils fairly large quantities of fruits. Penicilluim expansum Aspergillus niger, A. clavatus, etc. spoil the meat during storage and transportation. Fungi also causes

large number of diseases in human beings. Examples are- Aspergillosis, Blastomycosis, Moniliases, Cryptococcosis and Coccidiomyces. Fungi are well known for their destruction to agricultural crops, market and storage fruits and vegetable diseases. The list seems to be endless. They also cause destruction of substances like clothing material, leather goods, photographic materials, etc. Certain fungi causes destruction of wood either in furnitures and other materials or in standing trees.

Since a large number of plants are attacked by fungi and considerable loss is caused due to fungal pathogens, so it became desirable to survey the post harvest diseases of fruits and vegetables. For this purpose, the storage and markets were visited to collect the infected vegetables and fruits.

An extensive survey of the market of Allahabad and adjoining areas were made. The diseased fruits and vegetables were collected from the - Katra market, Khuldabad market, Meerapur market, Kareli Fruit market, Sabzi mandi, Chowk area, Mundera, Gau Ghat,

Sarai Aquil, Phaphamau as well as from local vendors. A large number of fungi were found to be associated with number of vegetables and fruits diseases in the market as well as in the storage. **[Appendix-I]**

Appendix-I : List of pathogens collected from different hosts during investigation.

	COMMON NAME	BOTANICAL NAME	FUNGI ISOLATED
1.	Apple	Pyrus malus	Alternaria sp.
2.	Aonla	Phyllanthus emblica	Fusarium semitectum, Pestalotiopsis sp.
3.	Banana	Musa paradisica	Botryodiplodia sp.
4.	Pear	Pyrus communis	Alternaria sp., B.themobromae.
5.	Carambola	Averrhoa carambola	Diplodia natalensis, Colletotrichum sp., B. theobromae.
6.	Mosambi	Citrus sinensis	B.theobromae, Alternaria sp., Penicillium sp.
7.	Orange	Citrus reticulata	B.theobromae, Aspergillus niger.

8.	Mango	Mangifera indica	Alternaria sp. B.theobromae, Colletotrichum gloeosporioides.
9.	Lemon	Citrus limon	Alternaria alternata
10.	Grapes	Vitis vinifera	Curvularia sp.
11.	Pineapple	Ananas comosus	B.theobromae, A.alternata
12.	Guava	Psidium guajava	Pestalotiopsis sp., Phoma sp.
13.	Papaya	Carica papaya	Alternaria sp., Rhizopus sp. Diplodia sp., Fusarium semitectum, Phoma sp.
14.	Custard apple	Anona squamosa	Fusarium semitectum Botryodiplodia sp., Colletotrichum sp.
15.	Pomegranate	Punica granatum	Aspergillus sp.
16.	Tomato	Lycopersicon esculentum	Alternaria sp., Cladosporuim sp., Collectotrichum sp., Fusarium roseum.

17.	Capsicum	*C. frutescence*	*Alternaria* sp., *B.theobromae*, *Curvularia lunata*,
18.	Brinjal	*Solanum melongena*	*Alternaria* sp., *Fusarium* sp.
19.	Potato	*Solanum tuberosum*	*Alternaria solani*, *Fusarium solani*,
20.	Radish	*Raphanus sativus*	*Alternaria solani*,
21.	Carrot	*Daucas carota*	*Alternaria solani*, *Fusarium culmorum*
22.	Pea	*Pisum sativum*	*Cladosporuim* sp., *Fusarium* sp.
23.	French bean	*Phaseolus vulgaris*	*Alternaria alternata*, Colletotrichum lindemuthianum
24.	Lablab Bean	*Dolichos lablab*	*Colletotrichum lindemuthianum*
25.	Onion	*Allium cepa*	*Alternaria* sp.
26.	Okra	*Abelmoschus esculentus*	*Curvularia* sp.
27.	Bitter	*Momordica*	*Fusarium* sp.

	gourd	charantia	
28.	Bottle gourd	Lagenaria siceraria	Fusarium sp., Curvularia lunata
29.	Pumpkin	Cucurbita moschata	Alternaria solani, Fusarium oxysporum
30.	Snake gourd	Tricosanthes cucumerina	Fusarium sp.
31.	Pointed gourd	Tricosanthes dioica	B.theobromae
32.	Ash gourd	Benincasa hispida	Fusarium sp.

A brief description of the fungi isolated during the practical work is as follows :-

ALTERNARIA : The genus stands distinct from other genera by its transversely -, and longitudinally-septate (muriform) conidia. The conidia show a distinct beak which may vary from very short to very long (equal to the conidia). The conidia (porospores) are usually formed acropetally in long chains, in some instances borne singly and having an apical simple or branched appendage. These are dark brown, typically obclavate with a distinct oval body and a beak. The

beak is light in colour, short or long and may serve as conidiophore. The conidiophores are dark, broader than the vegetative hyphae and is a sympodula. They bear geniculations marked with scars of detached conidia.

Neergard (1945), in his monograph on this genus, divided the species into three sections on the basis of the size of conidial chain or absence of the chain. The sections are Longicatenatae, with 10 or more conidia in the chain. e.g. A. tenuis; Brevicatenatae, with shorter chain of 3-5 conidia, e.g. A.citri and Noncatenatae, solitary conidia with long, filiform beak, e.g. A. solani.

Most of the species are parasitic causing leaf-spot diseases. A. solani causes "Early-Blight"of potato and other members of Solanaceae. The characteristic "target-board" - like leaf spots, show distinct concentric bands of light and dark colours.

The perfect stage is known for A. tenuis, the type species; it is Pleospora, of Ascomycotina.

<u>**ASPERGILLUS**</u> : The genus, though world wide in distribution, is more prevalent in tropical countries. The grains stored commercially below 13% of moisture content (equivalent to R.H. below 70%), are attacked by <u>A</u>. <u>glaucas</u>, <u>A</u>. <u>halophilicus</u> and <u>A</u>. <u>restrictus</u> and are thus, called 'storage fungi' (**Christensen**, 1965). The hyphae are hyaline, septate and branched. The cells are mulinucleate. The conidial stage is dominant and the sexual stage is either absent or rarely produced. Species which have only conidial stage are called <u>Aspergillus</u> while those in which sex organs are formed are placed in the genera <u>Eurotium</u>, <u>Emericella</u> and <u>Sartorya</u>. Some species are weak parasites causing diseases of fruits during storage and transit.

The mycelium starts forming conidiophores very early. The long, simple, erect and upright conidiophores arise from a particular thick walled foot-cell. It forms a terminal swollen vesicle, on which bottle-shaped structures called <u>sterigmata</u> or <u>phialides</u>, arise at the apex or radiating from the entire surface. They produce chains of conidia at their tips. When there are 2 layers of sterigmata, the

lower is called primary sterigmata or metulae and the upper, as secondary sterigmata or phialides. The conidia (phialospores) are 1-celled, globose, circular, multinucleate and have a thick and rough wall. They are often variously coloured in mass. The conidia remain attached in chains.

The colour of the conidiophores and the conidia are responsible for the colour of the colony, which may be green (*A. flavus*), yellow (*A. ochraceus*), brown (*A. tamari*), black (*A. niger*), white (*A. candidus*) etc. The colony colour is an important criterion in the identification of species.

BOTRYODIPLODIA : The pycnidia lie aggregated in botryose (grape-like) clusters, hence the name *Botryodiplodia*. The pycnidia are dark brown or black in colour. They are thick-walled, ostiolate, erumpent, stromatic and confluent. conidiophores are simple, short, cylindrical and hyaline. They show distinct apical annelations. The conidia are dark-brown, ovoid to elongate with characteristic striations at maturity. These are 2-celled at maturity. Young

conidia are 1-celled and hyaline. They are parasitic or saprophytic on twigs. This genus is much like Macrophoma or Dothiorella, if only immature conidia are present. B. theobromae is the most common species which causes fruit rot of mango, cacao, etc.

BOTRYTIS : B. cinerea, the "grey mold", is the best-known species. It is the conidial stage of Sclerotinia, a Discomycete (Ascomycotina). It commonly attacks strawberries, covering it with a grey moldy mass. **Brown** (1915-1922) used this organism in his studies on the physiology of parasitism and described the production of enzymes pectinase and protopectinase in host during disease development, which brought about tissue disintegration.

The conidiophores are brown, long, slender, simple and erect. They are variable in length and becomes hyaline and irregularly branched towards the apex. Sometimes they are pigmented. The ultimate branches bear the densely-aggregated conidia or botryoblastospores. Each conidium is borne on a minute sterigmata. The conidia are ash-coloured or hyaline,

unicellular, ovoid, apiculate at base, dry and easily blown away. In culture, sclerotia appear after a few days, which are irregular in shape and serve to perennate the fungus. They are parasitic, causing "grey mold" of many plants or are saprophytic.

CLADOSPORIUM : Conidiophores are tall, dark, upright and branched variously near the apex. They may be single or clustered. The conidia (blastospores) are dark, 1 or 2 called, variable in shape and size, ovoid to cylindrical and irregular. Some are typically lemon-shaped. They are often before in simple or branched acropetalous chains. The blastospore or conidia is formed by simple budding as in yeasts. A narrow zone of the conidiogenous cell blows out, enlarges and develops into a conidium. A constriction is conspicuous between the conidium and the conidiogenous cell before separation. They are parasite on higher plants or are saprophytic.

COLLETOTRICHUM : Colletotrichum causes 'anthracnose' (coal-like) leaf-spot diseases of several crops, and is, therefore, known as the anthracnose fungus.

Acervuli may be sub-cuticular, subepidermal or epidermal. They are disc-shaped, waxy, with a characteristic peripheral ring of black, long and stiff spines or setae. Conidiophores are simple and elongate conidia are hyaline, 1-celled, ovoid or oblong. They are imperfect stages of *Glomerella* and are parasitic. The gums differs from *Gloeosporium* in having spines, which may be absent in certain cultural conditions. Some important pathogenic species are - *C.falcatum*, the cause of "red rot" of sugarcane; *C. corchorum*, causing anthracnose of jute; *C. gloeosporioides* causing anthracnose of citrus, *C.graminicola,* anthracnose of jowar; *C. musarum*, anthracnose and fruit rot (cigar end disease) of banana, *C. falcatum* is now proved to be the asexual stage of *Glomerella falcatum* and *Physalospora tucamanensis*, both belonging to sphaeriales of Ascomycotina.

CURVULARIA : Species of *Curvularia* are common leaf-spot fungi and also occur in soil. Some are active degraders of cellulose and damage textile.

The conidiophores forms a sympodula bearing a cluster of conidia. They are brown, mostly simple, bearing spores apically or on new sympodial growing points. The detached conidium leaves a knob-like scar on the conidiophore on the 'bends' or geniculations formed due to the characteristic growth as a sympodula. The conidia or porospores are dark brown 3-5 celled, more or less fusiform, typically bent and mostly curved about the third cell from the base (hence the name <u>Curvularia</u>), which is also much larger and darker in colour than rest of the cells. They may be parasitic or saprophytic. Perfect stage of <u>C. lunata</u> is <u>Cochliobolus lunata</u>. The species of <u>Curvularia</u> are segregated into three groups-maculans, lunata and geniculata conidia 3-septate.

Conidia mostly straight or feebly curved with the central two cells larger and darker than the end cells, broadest in the middle - <u>maculans group.</u>

Conidia distinctly curved, rarely straight with one of the central cells larger and darker than the

other cells, broadest above middle - lunata group

Condia 4 or more - septate - geniculata group

DIPLODIA : Diplodia forms solitary, black, globose, erumpent, ostiolate and immersed pycnidia. The conidiophores are simple and slender. The conidia are dark brown, 2-celled, ellipsoid or ovoid. The species may be parasitic or saprophytic. D.zeae colonizes dead maize stalks while D.macrospora caused dry-rot of maize ears and stalks.

FUSARIUM : Fusarium is a facultative parasite occurring commonly in the soil as saprophyte. The genus is an important plant pathogen causing "damping-off" of seedlings, 'root rot' and 'wilt' of various economically important plants. It is more prevalent in tropical countries. It is a large and variable genus, sometimes placed in the Tuberculariaceae because some species produce sporodochia.

The mycelium is extensive and cottony in culture, often with some tinge of pink, purple or yellow. The mycelium is made up of much branched, septate and multinucleate hyphae. The hyphae are

restricted to the vascular tissues and are both intercellular and intracellular. The fungal hyphae form matted coils within the vascular tissues of the root and basal portion of stem which result in the plugging of the lumen of the xylem vessels. The plugging of the xylem vessels by the fungus hyphae interfere with the free flow of water to the leaves which consequently wilt and droop. The fungus hyphae also secrete certain toxins mainly the fusaric acid which are toxic to the living cells in the tissues concerned in the ascent of sap.

The conidia are formed in slimy, effuse sporodochia called pinnotes, or sometimes scattered on the mycelium. The conidiophores are variable. They may be slender and simple, or stout and short. Conidiophores are branched irregularly or bear a whorl of phialides, single or grouped into sporodochia. The phialides are subulate i.e. widest at the base, narrowing to a point like on 'awl'. The conidia (phialospores) are hyaline, variable, principally of two kinds - The microcondia which are 1-called, ovoid or oblong, pyriform or elongate and are borne singly

or in chains. The macroconidia which are several-celled, hyaline or pale, slightly curved or bent at the pointed ends, typically canoe shaped; hence the name fusus = spindle. The macroconidia with a distinct "foot cell", bearing some kind of 'heal', is the most district feature of Fusarium. Chalamydospores, if present, are formed on the hyphae or on the macroconidia. Microconidia may be present or lacking. The conidia are formed in succession and become enveloped in a slimy mass. In addition to conidia and chalamydospores, some species form sclerotia which are brightly coloured. When the conditions are favourable, the conidia or chalamydospores germinate by means of germ-tube into new mycelium. The perfect stages of the various species belong to several ascomycetous genera, viz. Hypomyces, Gibberella, Nectria and Calonectria. Fusarium is a problematic genera due to its great variability.

PENICILLIUM : The species of Penicillium are cosmopolitan in distribution, but are more prevalent in temperate countries. the species of this genus are saprophytic, that are abundant everywhere. They are

commonly known as blue moulds or green moulds. Decaying oranges, lemons and other fruits show a bluish growth on them, which is due to the blue coloured spores of Penicillium. Various species of Penicillium are responsible for economic loss. Blue mold of citrus fruits (P. italicum), green mold of citrus fruits (P. expansum) are some of the important diseases caused by Penicillium. Several other species produce toxins on food stuffs which causes toxaemia in man and animals. P.notatum and P.chrysogenum produce the best known antibiotic, called penicillin.

The mycelium consists of highly branched and septate hyphae. The mycelium may grow superficially forming a welt upon the substrate or penetrate deeply into the substratum for absorbing the nourishment. The characteristic conidial apparatus which resembles a brush or broom, is called a 'penicillus' (penicillium= small brush). Long, septate conidiophores arise from any cell of the hyphae and not from a specific foot cell as in Aspergillus. The conidiophores branch once or twice at two-third of its total length. These branches are called primary sterigmata or rammi (sing.

ramus) and secondary sterigmata (=metulae), which finally bear the bottle-shaped phialides. The species are chiefly based on the nature of branching of the conidiophore which may be mono-, or biverticellate. In the manoverticellate condition, the phialides are borne directly on the conidiophore while in biverticellate, these are borne on a further whorl of branches called metulae which in turn may arise on a whorl of branches called rammi. Conidia or phialospores are produced in the neck region of the phialide. Long chains of conidia are formed with the youngest conidium at the base i.e. basipetal chains. The conidia are hyaline or brightly coloured in mass. They are 1-celled and globose to ovoid and look like glass beads under the microscope. The coloured conidia- green, blue or yellow give the characteristic colour to the colony, which aid in identification of species. Each conidium under favourable conditions, germinates by a germ tube into a new mycelium.

PHOMA : Phoma is similar to Phyllosticta. The pycnidia are dark, and ostiolate. They are lenticular to globose and are immersed in host tissue. They may be

erumpent or with a short beak piercing the epidermis. The conidiophores are short. The conidia are small and hyaline. They are 1-celled, globose to oval, and guttulate i.e. have one or more oil drops inside. The conidia of Phoma differ from Phyllosticta in lacking the minute apical mucilagenous appendage. The conidia in some species of Phoma are phialospores, originating from monophialides.

PESTALOTIA : The genus is unmistakable because of the characteristic shape of its conidia. The conidia are spindle-shaped or clavate, several-celled (or 5-celled) with the three central coloured cells and two (upper and lower) hyaline cells. The upper hyaline cell bears 2 or 3 cellular apical appendages called metulae, which in some species are branched. There is a short hyaline pedicel below the lower hyaline cell. Some species are destructive pathogens. e.g. P.theae, causing 'grey-blight' disease of tea leaves, and T.mangiferae, causing leaf spot of mango.

PATHOLOGICAL STUDIES

If you find an organism misbehaving in the field,

don't spray it, don't fumigate it,

investigate it!

[R.G. Grogan]

Like all living beings, fruits and vegetables are subject to a number of diseases incited by micro organisms. the diseases of fruits and vegetables which develop during the post-harvest phase are of considerable importance.

The chemical composition and nutritive value of the fruits and vegetables may be influenced by a number of biological entities viz., fungi, bacteria and viruses. According to **Smith** et. al. (1964). "There are more than 250 known parasitic diseases of fruits and vegetables that cause decays and blemishes during the marketing process. The damage and losses incurred vary with the crop, growing conditions in the field, handling during harvest and transit, and storage conditions. At present the number of post harvest diseases has increased several folds. According to

Mehta, 1975, these diseases are now responsible for 20-30% loss of the crops annually.

Of all the diseases caused by micro-organisms, fungi are responsible for heavy damage to fruits and vegetables. Losses may occur during their shipment, storage and marketing processes. These may be due to infection in the field or during harvesting, packing and transit processes. **Stevens** and **Stevens** (1952) mentioned, "of all losses caused by plant diseases, those which occur after harvest are most costly, whether measured in monetary terms or in men hours.

A Loss estimation is a tedious task and in perishables the estimations are not as precise as they are in the durable commodities. The losses are highly locality specific and level of loss acceptable in rural market differs greatly from that acceptable in commercial post-harvest sector. Nevertheless, experts with long experience in the field have estimated 20-30% losses in fruits and vegetables under Indian conditions. (**Anon.**, 1977). The loss estimation for onion (16-35%), tomato (20-50%), cabbage (37%), Cauliflower (49%), and lettuce (62%) in developing

countries have been reported (**Anon**, 1976). The most common pathogens causing rots in vegetables and fruits are fungi such as Alternaria, Botrytis, Diplodia, Monilinia, Phomopsis, Penicillium, Rhizopus, and Fusarium. While most of the pathogens can invade only the damaged tissue, a few such as Colletotrichum are able to penetrate the skin of healthy tissue.

Different disease problems arise when crops are harvested because the seeds, fruits or other storage organs are essentially dormant structures and their cells are physiologically unlike those of the growing plant. Therefore, the bulking of these structures in transit and storage poses problems quite different from those encountered in the field.

Soil infesting fungi that cause loss of fleshy tissues typically infect plants at the time of, or just before, harvesting. Infection may occur, however, during post harvest handling or storage. All important rot pathogens initiate disease in the same general way. They all produce extracellular enzymes and start the degenerative process in advances of the fungal hyphae of the attacking pathogens. Infact, these

organisms are all saprophytic pathogens and obtain nourishment from dead or dying cells. The observation of **Simmonds** (1963) in connection with the infection of fruits of bananas is very relevant. According to **Simmonds**, the ripened fruits are infected easily, while the green fruits show resistance to infection.

As a result of infections, the market and nutritive value of the fruit is lowered, either due to its ugly appearance or the changes in the stored products of the fruits, vegetables and seeds.

A large number of fungi are responsible for storage diseases of fruits and vegetables. Rhizopus spp. cause considerable loss to peaches, grapes, strawberries, sweet potatoes, cucurbits, crucifiers, tomatoes and egg plants. Rhizopus spp. produce soft rot of the fleshy parts which proceeds rapidly at high temperatures. There is often a leakage of juices from the affected parts of fleshy fruits and vegetables. In temperate parts, stone and pome fruits are affected by brown rot fungi such as Sclerotinia fructigina, S. laxa, and S. fruiticola. Bananas in storage and

transit are subjected to various fungal rots (**Wardlaw**, 1961). Rotting is generally brought about by Gloeosporium musarum. Penicillium spp. cause considerable damage to citrus fruits in storage and transit (**Rose** et al., 1943). Potato tubers are commonly attacked by Fusarium caeruleum. Species of Aspergillus are very often responsible for the deterioration of stored grains (**Christensen** and **Kaufman**, 1965).

In our own country, due attention has been paid to investigate the post-harvest diseases. Earlier the plant pathologists were mostly concentrating on the study of the diseases of fruits and vegetables under field conditions. But now extensive research work is going on in this field. But still in most cases, the causal organisms and their symptoms have only been listed and no detailed work has been carried out on their physiological and pathological behaviour. However, there are several worthementioning contributions from India in this field. Some of the important contributions are that of **Mitter** and **Tandon** (1929) and **Mehta** (1937) on diseases of apples and

pear, **Chona** (1933) on diseases of banana, **Ghaṭak** (1938) on orange rots, **Sinha** (1946) on storage diseases of mango, apple, pear, peach, orange, grape fruit and pomegranate, etc., **Bhargava** and **Gupta** (1957) on market diseases of certain fruits and vegetables in Kumaon, **Damodaran** and **Ramakrishnan** (1963) on anthracnose of banana, **Tandon** and **Verma** (1964) on certain new storage diseases of fruits and vegetables, **Rao** (1964, 1965, 1966) on market diseases of fruits and vegetables in Bombay-Maharashtra, **Srivastava** et al. (1964, 1965, 1966) on post-harvest diseases of some fruits and vegetables, **Dastur** (1916) on rot of bananas, etc.

Despite of the above mentioned works and recent advances in this field, more investigations on fruit and vegetable rots and their possible control measures are required. Thus realising the importance of post-harvest diseases an attempt has been made to undertake a detailed study of some important post-harvest diseases of some fruits and vegetables.

Symptoms of the various diseases have been described and the morphological characters of the

isolates, have been given below. Only pathogenic forms have been included.

Anona Squamosa L. (Custard apple)

(1) Botryodiplodia rot of Custard apple :

Symptoms - The infection begins at the stem end which progresses downwards and cover the whole fruit. It is characterized by the softening of pulp. In the advance stages of disease the rind turns dark brown or black. It gives unpleasant odour.

Causal organism: *Botryodiplodia theobromae*

(2) Anthracnose of custard apple :

Symptoms - In the beginning the disease in present in the form of small angular, brown to black, cankerous spots which later coalesce to form big lesions. In later stages, minute dot like acervuli appears. The infection is not very deep.

causal organism: *Colletotrichum* Sp.

Ananas comosus (Pineapple)

(1) Botryodiplodia rot of Pineapple :

Symptoms - The infection begins at the distal end. It is characterized by the softening of the host tissue. It is characterized by brown colour infection. The infection is slow in the beginning causal organism: Botryodiplodia theobromae.

(2) Alternaria rot of Pineapple :

Symptoms - In the initial stage, small, irregular dark brown to black coloured spot appear in the beginning. Gradually they enlarge and cause a penetrating decay.

Causal organism: Alternaria alternata.

Averrhoa Carambola (Carambola)

(1) Botryodiplodia rot of carambola :

Symptoms - The disease begins at the stem end. The infection is shown by brownish discolouration. It gradually extends to the other end along with ribbed

margins. During later stages, pycnidial initial are formed.

Causal organism: B. theobromae

(2) Anthracnose of Carambola :

Symptoms - The infection starts with the light brown pits produced on the margins of the fruits. Later these spots enlarges. Ultimately spores oozed out from the acervuli.

Causal organism : Colletotrichum gloeosporioides

Carica papaya L. (Papaya)

(1) Fusaruim rot of papaya :

Symptoms - The rot starts near the stem end of the fruit. It is usually brownish spot, which is slightly soft. Later it is covered with white fluffy growth.

Causal organism : Fusarium semitectum

(2) Phoma rot of Papaya :

Symptoms - The disease is characterized by the appearance of one or few slightly sunken, light brown spots. Later they coalesce, get depressed and attain an irregular shape. In more later stages, the pycnidia start appearing. The decay penetrates deep into the pulp turning the tissue dark brown to black in colour.

Causal organism : *Phoma* sp.

(3) Alternaria rot of Papaya:

Symptoms - The disease in characterized by the production of brown coloured spots, which are irregular in shape. These are sunken and surrounded by yellow halo. Gradually they get covered by dense, velvety, black conidial masses. The rot extends deep into the pulp.

Causal organism: *Alternaria* sp.

(4) Rhizopus rot of papaya :

Symptoms - The disease appears in the form of a water soaked spot on the skin which enlarges rapidly

covering almost the whole fruit. The tissue becomes soft and watery. Finally, the fruit is covered with white or grey mould.

Causal organism: Rhizopus sp.

Citrus aurantifolia (Lime)

(1) Fusarium rot :

Symptoms - The rot begins near the stem end of the fruit. It is brown in colour and slightly soft in nature. Later the fruit gets covered with white growth.

Causal organism : Fusarium sp.

Citrus sinensis (Sweet Orange)

(1) Alternaria rot:

Symptoms - The disease, is popularly called black rot (**Pierce**) 1901, 1902). It is characterized by the production of black decay around the button or at the stylar end. The decay process is usually slow. The

affected tissues turns black and the rind is dark brown to black in colour.

Causal organism: *Alternaria* sp.

(2) **Blue and green mould rots** :

Symptoms : Initially the disease occur in the form of soft watery discoloured spots on the rind. Under favourable conditions they enlarge and then the blue mould produces dull blue colonies surrounded by narrow white mycelial growth. The green mould produces fast growing colonies, usually surrounded by a wide zone of white mycelial growth.

Causal organisms : *Penicillium italicum* (blue)

P. digitatum (green)

Citrus reticulata (Orange)

(1) Aspergillus rot :

Symptoms - The disease appears in the form of very soft light brown spot. later, the decayed area gets depressed and is covered by the black mouldy

growth of the fungus. The healthy tissue is clearly demarcated from the infected region.

Causal organism : *Aspergillus niger*

(2) Stem-end rot :

Symptoms - It begins at the stem end and is characterized by softening of the rind and underlying pulp. Subsequently the affected rind turns brown or dark brown. The infection progresses rapidly and reaches the blossom end. The diseased tissue is soft and watery in nature.

Causal organism: *Botryodiplodia theobromae*

Lycopersicon esculentum Mill. (Tomato)

Alternaria rot :

Symptoms - It begins with the production of brown coloured spots. They are sunken and irregular in shape. Gradually they get covered by dense, velvety, olive green or black conidial masses. The lesions are firm. They extend deep into the tissues and produce scanty fungal growth.

Causal organism : *Alternaria* sp.

(2) Anthracnose :

Symptoms - In the beginning the lesions are small, circular, slightly depressed and water soaked. Later they increased in size, become darker and produce cream to salmon coloured spores in the centre which later appears as dark spots. Gradually the spots increase in size and penetrates deep into the pulp.

Causal organism: *Colletotrichum* sp.

(3) Cladosporium rot :

Symptoms - The disease manifests itself as a small, light brown spot which later enlarges. The centre of the lesion attains a dark brown colour. The rot is usually limited to the outer wall of fruit. Generally, the decay process is slow.

Causal organism: *Cladosporium* sp.

(4) Fusarium rot :

Symptoms - The rot begins with the production of a water-soaked lesion, which later becomes shrunken due to softening and wrinkling of the affected

tissues. The area is slightly raised and is whitish to pinkish in colour. The decay progresses rapidly and cause soft disintegration of the pulp.

Causal organism: *Fusarium roseum*

Mangifera indica L. (Mango)

(1) Aspergillus rot :

Symptoms - The disease begins at the stalk end. In the initial stage, light brown circular spot develops which grows from the centre of the stalk in a regular pattern. Later it shows the softening of the pulp. Finally, black conidial heads of *Aspergillus* appear. Besides causing stalk end rot, *Aspergillus* also causes lateral rot, tip-end rot or a rot at any other place on the fruit.

Causal organism: *Aspergillus niger*

(2) Botryodiplodia rot :

Symptoms - The infection appears at the stem end and progress downwards in an irregular manner. It develops a water-soaked lesion. The upper part of the

fruit acquires a dark brown colouration but the peripheral region remains light brown. The margin is wavy. The rot is soft and watery.

Causal organism : Botryodiplodia theobromae

(3) Colletotrichum rot or anthracnose spot :

Symptoms - The disease begins with small brown circular spots on the surface of fruits. The spots sometimes coalesce to for larger irregular spots of dark brown colour. The decay is usually confined to the skin but sometimes it may penetrate the mesocarp.

Causal organism : Colletotrichum gloeosporioides

Musa paradisica L. (Banana)

(1) Botryodiplodia rot :

Symptoms - The disease manifests itself as a brownish and water-soaked discolouration. It proceeds irregularly on either end. Generally the rotting of the pulp is faster as compared to that of the rind.

Causal organism : B. theobromae

Emblica officinalis (A'onla)

(1) Pestalotiopsis rot :

Symptoms - The disease appears in the form of brown coloured spots with irregular margin over the surface of the fruit. They later on enlarges and get covered with fluffy growth of the fungus.

Causal organism: *Pestalotiopsis sp.*

Dolichos lablab L. (Lablab or Indian bean)

(1) Anthracnose :

Symptoms - The disease is characterized by the presence of numerous small, oval to circular, brown, sunken spots. Very often they coalesce to form big lesions with reddish brown border.

Causal organism : *Colletotrichum lindemuthianum*

Pisum sativum L. (Pea)

(1) Scab :

Symptoms - Dark brown or black, irregular, raised spots are produced on the surface of the pods.

The inside of the wall beneath the scab lesions usually shows white felty or hair-like proliferations.

Causal organism : *Cladosporium* sp.

(2) Fusarium rot :

Symptoms - The rot usually begins as a brownish spot which is slightly soft in nature. Later it becomes covered with whitish growth of the fungus.

Causal organism : *Fusarium semitectum*

Psidium guajava L. (Guava)

(1) Pestalotiopsis rot :

Symptoms - The disease begins with the brownish discolouration which after few days attains a rust colour. Later the spots enlarges and gets depressed. Subsequently black, minute acervuli with spore masses apear.

Causal organism: *Pestalotiopsis* sp.

(2) Diplodia rot :

Symptoms - It begins at the stem end and is characterized by softening of the rind and the pulp. Subsequently the affected rind turns brown. The infection progresses rapidly and reaches the other end. Affected fruits do not show fungal growth unless kept under moist conditions. The diseased tissue is watery in nature.

Causal organism : *Diplodia* sp.

(3) Phoma rot :

Symptoms - Brown coloured circular spot is produced over the surface of the fruit. The centre gets depressed and the water-soaked margin shows sparse fungal growth. The minute pycnidia produces cream coloured spores.

Punica granatum L. (Pomegranate)

(1) Aspergillus rot :

Symptoms - The disease begins with brownish discolouration. Gradually it becomes blackish and somewhat depressed. Later it get covered with green spores.

Causal organism : Aspergillus sp.

Pyrus communis L. (Europ. Pear)

(1) Stem - end rot:

Symptoms - Brown coloured necrotic lesions are produced at the stem end of the fruit. In advanced stage pycnidial bodies appear.

Causal organism : b. theobromae

(2) Alternaria rot :

Symptoms - The disease appears as small, irregular brown spot. It gradually becomes dark brown, leathery and sunken. Later it shows a sort of concentric markings.

Causal organism : Alternaria Sp.

<u>Solanum melongena</u> L. (Brinjal)

(1) <u>Alternaria rot</u> :

<u>Symptoms</u> - In the initial stages, small irregular brown coloured spot appear in the beginning. Gradually they increase in size and penetrates causing the decay.

<u>Causal organism</u> : <u>Alternaria</u> sp.

(2) <u>Tip-end rot</u> :

<u>Symptoms</u> - Infection usually starts from the tip end. The infected tissues become brown and get shriveled forming a dry rot.

<u>Causal organism</u> : <u>F. roseum</u>

<u>Vitis vinifera</u> L. (Grapes)

(1) <u>Curvularia rot</u> :

Symptoms - The diseased fruit showed brown, circular spot. It gradually turns black and cottony mycelium appeared which is black due to the production of spores.

Causal organism : Curvularia sp.

Benincasa hispida (Ash gourd)

(1) Fusarium rot :

Symptoms - This rot begins with production of water soaked lesion, which is some what shrunken due to the softening of the tissues. The fungus often appears early at the centre of the spot. The area is slight pinkish in colour. Gradually it causes disintegration of the tissues.

Causal organism : F. roseum

Tricosanthes dioica L. (Pointed gourd)

(1) Stem-end rot :

Symptoms - The disease begins with the formation of brownish discolouration at the stem end.

Gradually it progresses and penetrates deep into the tissue causing a soft rot.

Causal organism : B. theobromae

Abelmoschus esculentus L. (Lady's finger)

(1) Curvularia rot :

Symptoms - The infected fruit showed small circular or oval light brown coloured spots. It gradually extend towards both the ends of the fruit and turns black. Later cottony mycelium which turns black appeared.

Causal organism : Curvularia sp.

Tricosanthes cucumerina L. (Snake gourd)

(1) Fusarium rot :

Symptoms - The infection starts at the tip end usually. The infected tissue get brown colouration. It shriveled and dry rot is developed.

Causal organism : Fusarium sp.

Pyrus malus L. (Apple)

(1) Alternaria rot :

Symptoms - It begins with the appearance of small irregular brown coloured spot. Gradually they enlarge with an irregular margin and penetrates deep into the pulp. In advance stage the whole fruit is rotten due to the deep penetration of pathogen into the fruit.

Causal organism : *Alternaria* sp.

STUDY OF SPORES

Spores, are the basic unit of reproduction in fungi. They are specialized microscopic structures produced at the climax of their life cycles. This is evident that the dissemination and multiplication of spores are responsible for the propagation and occurrence of various diseases. They are also

responsible for the survival of the pathogens in nature.

According to **Gottlieb** (1964), "Germination is the process by which a spore is transformed from a dormant state of low metabolic activity to one of high activity. Formation of the germ tube is the outward and visible sign that the metabolic change is complete." Germination of fungal sores is essentially a process during which the normal metabolic and physiological activity is restored after a temporary halt or check in these activities in the resting spores, followed by a morphological transformation of the spore into a thallus. The method of germination of different kind of spores varies. In most fungi it is accomplished by the formation of a germ tube, which continues to elongate and ultimately leads to the formation of the vegetative body of the fungus.

Spore germination is influenced by two factors (**Wolf** and **Wolf**, 1947, pg. 211): hereditary or internal and environmental or external. These include dormancy, maturity, longevity, water nutrients, temperature and hydrogen ion concentration, etc. which inhibit or

accelerate germination of spores. A study of various factors influencing the germination of spores may be helpful in understanding the ecology of pathogen as well as the physiology of the infection processes.

An attempt was, therefore, made to study the spores of A. alternata, F. oxysporum and B. theobromae under the following heads :-

(1) Mode of Spore germination.

(2) Effect of some external factors on the germination of spores -

(i) Effect of water and some nutrient solutions

(ii) Effect of different temperatures.

(iii) Effect of different hydrogen-ion concentrations.

(3) Determination of thermal death point.

(1) Mode of Spore Germination : To study the spore germination of A. alternata, hanging drop cultures

were prepared and they were kept under constant observation. The spores of *A. alternata* swelled up due to the absorption of water. The protoplasmic contents became visibly distinct after ½ an hour. After about two hours, the germ tube protuberances became visible. After 3-4 hours the germ tube made its appearance and increased in size as time went on. It took almost 3 and a ½ hours. Finally it gave rise to branched hyphae.

When the spores of *F. oxysporum* were placed in water, they absorbed water and swelled up. Their cellular contents became more distinct. Germ tube appeared initially as a small protuberance from the spore and 4-5 hrs were required for this. Later they developed into a branched mycelium.

The spores of *B. theobromae*, absorbs water and swelled up. Their protoplasmic contents became more distinct. It took about 2½ hrs for the germ tubes to appear as a small protuberance. This protuberance finally enlarged and gave rise to a branched septate hyphae. Sometimes the hyaline, aseptate but nearly mature spores also gave out the germ tube.

(2) Effect of Some External factors on the germination of spores :

(i) Effect of water and some nutrient solutions :

It is presumed that most of the nutrients required for germination of spores are present in themselves. However, several workers have, shown that an exogenous supply of nutrients is essential for initiation of spore germination. According to **Lilly** and **Barnett** (1951, p.361), "Some species germinate well in distilled or tap water, while others require certain special nutrients such as sugar, salts or even a particular nitrogen source. No one medium has been found which will allow good germination of all fungi, although certain natural media, such as beet or bean decoction and soil infusion, seem to favour germination in a large number of fungi."

Therefore, in the present study the following solutions (sterilized) were used to study their influence on spore germination of the three fungi under study - tap water, distilled water, 1% glucose

solution, basal medium and host extract. The results are given in table - 1

Table-1: Effect of different nutrient solutions on the percentage germination of spores of the three fungi after eight hours of incubation.

Sl. No.	Nutrient Solutions	Percentage of Spore germination		
		A. alternata	*F. oxysporum*	*B. theobromae*
1.	Tap water	46.0	28.0	35.0
2.	Distilled water	47.0	40.0	60.0
3.	1% glucose	84.0	70.0	76.0
4.	Basal medium	85.0	83.0	85.0
5.	Host decoction	92.0	89.0	96.0

From the table-1, it is clear that the spore germination of all the 3 fungi under investigation was minimum in tap water, whereas it was maximum in host extract solution. Basal medium ranked the next best nutrient solution. The percentage of spore germination was less in distilled water as compared to other nutrient solutions. Lack of nutrient in distilled

water was responsible for poor germination of spores. Minimum germination in tap water was probably due to the presence of chlorine.

Thus it is clear that although the presence of external supply was not necessary for germination, yet it exhibited profound effect on percentage germination of spores in all the three cases.

(ii) Effect of temperature; Since the atmospheric temperature determines the seasonal spread of the disease, so it was essential to study the effect of different temperatures on the germination of spores of the pathogens under study. The observations are summarised in table - 2.

From the Table - 2. It is evident that the maximum percentage of germination was observed in all the pathogens under study at 25^0C, which was followed by germination at 30^0C and 20^0C. All the three pathogens showed nil spore germination percentage at 5^0C and 40^0C.

Table-2: Effect of different temperatures on percentage germination of spores of the organism under study after incubation for eight hours.

Temperature in ^{0}C	Percentage of Spore germination		
	A. alternata	F. oxysporum	B. theobromae
5.0	-	-	-
10.0	32.0	40.0	20.0
15.0	55.0	45.0	43.0
20.0	75.0	80.0	76.0
25.0	97.0	95.0	83.0
30.0	80.0	82.0	80.0
35.0	68.0	50.0	43.0
40.0	-	-	-

(iii) Effect of hydrogen ion concentration : The pH of the medium has been known to influence the germination of fungal spores. Generally, the spores germinate within a wide pH, yet an acidic medium is favourable for the germination of most species of fungi (**Lilly** and **Barnett**). According to **Cochrane** (1958, p. 403), "Spores of most of the fungi germinate favourably at pH 4.5 to 6.5." Hence, the effect of

different pH on spore germination was undertaken and the results are recorded in table-3.

Table-3: Percentage germination of spores of three fungi understudy at different pH levels after eight hours of incubation.

pH	Percentage of spore germination		
	A. alternata	*F. oxysporum*	*B. theobromae*
2.0	-	-	-
3.0	8.0	10.0	14.0
4.0	11.0	20.0	24.0
5.0	52.0	55.0	69.0
6.0	88.0	89.0	90.0
7.0	51.0	55.0	67.0
8.0	30.0	40.0	47.0
9.0	12.0	19.0	21.0
10.0	-	-	-

Table-3. indicates that all the three pathogens under study failed to germinate at pH 2.0 and 10.0. There was an increase in germination percentage between the pH range 3.0 to 6.0. However, pH range

over 6.0 showed gradual decline in germination percentage of spores of the pathogen under investigation.

(3) Determination of Thermal Death Point :

Cochrane (1955, pp. 423-424) defined thermal death point as the least temperature at which all the cells are killed in ten minutes. Many investigators, including **Faull** (1930),**Williams** (1941), **Yarwood** et al. (1954), **Goos** et al. (1961), **Prasad** (1963) and **Williamson** (1964) have determined the thermal death points of a large number of fungi.

The thermal death point throw light on the survival of the pathogen in nature and in storage. A knowledge of the thermal death point of the three pathogens may be helpful in suggesting suitable treatment for prevention of rots caused by them. Hence the thermal death point of each pathogen was determined by exposing its spores at different temperatures for 10 minutes. The results are recorded in table-4.

Table-4: Showing range of temperature at which an exposure for ten minutes killed the spores of organisms under study.

Temp. (^{0}C)	*A. alternata*	*F. oxysporum*	*B. theobromae*
50.0	+	+	+
55.0	+	+	+
60.0	–	–	–
65.0	–	–	–

The results from the table-4 shows that all the three pathogens were killed above 55^{0}C temperature when their spores were exposed for 10 minutes. A review of literature revealed that the thermal death point of most of the fungi lies between 40^{0}C and 60^{0}C.

ENVIRONMENTAL STUDIES

Freedom to inquire into the nature of things is a rewarding privilege granted to a few by a permissive society.

[Anonymous]

It has long been realized that plant diseases are greatly influenced by the environment **Theophrastus** (370-286 B.C.) believed that the position and character of the land made a considerable difference with respect to attack of rusts on cereals. He believed that crops growing on elevated land exposed to the wind were not liable to rust, or were less so than those growing on lowlands not exposed to wind. It was accepted at that time that microorganisms associated with diseased plants arose spontaneously from the plants or possibly from the environment. This theory of spontaneous generation dominated the thoughts of those studying plant diseases for more than 2000 years.

Human welfare, disease and weather have been associated together for ages. The ancient greeks associated the wind and disease. **Alexander** the Great

observed that when he camped near a swamp, a swamp disease appeared to come with the wind.

It is just within the past few years that there has been any real attempt to make a careful study of the factors of weather and their effect on plant disease. It has been recognized for a long time that the spores of fungi are well adapted for wind and water dispersal.

Some workers during the eighteenth century did not accept the almost universally held theory of spontaneous generations; one of these was **Joseph Pilton de Fournefort** (1705). He foresaw that fungi are autonomous organisms rather than things that arise by spontaneous generation. He also recognized that they could induce diseases in plants but was unable to provide experimental proof that this was so. Almost another hundred years passed before **Prevost** (1807), in his classic study of wheat bunt, demonstrated experimentally that the cause of the disease was a fungus.

Prior to **Prevost**, opinion as to the cause of wheat bunt varied. **Tillet** (1735) attributed wheat bunt to such factors as fog, wind, excessive soil moisture, improper plant nutrients, and soil texture. **Tesier** (1783) attributed the cause of cereal rust to mist blocking the transpiration of the plant. **Unger** (1833) believed that fungi associated with diseased plants were not parasites, that they had no independent existence, and that under certain environmental conditions every plant had the potentiality of producing them from the waste material of the plant. Acceptance of the idea that fungi can induce plant diseases, as shown by **Prevost** for wheat bunt, came only as a result of the contributions of **Berkeley** (1846,1847) on potato blight and vine mildew and the proof by **Speerschneider** (1857) and **De Bary** (1861) that the fungus Phytophthora infestans is the actual cause of potato blight. Although **Kühn** (1858) accepted the nature of fungi as pathogens, he continued to believe that environmental conditions could cause some diseases. **Hallier** (1868) emphasized the influence of soil, and the predisposition of the plant.

One of the first plant pathologists to appreciate the significance of the environment in relation to disease was **Hartig** (1882). He stated that a plant contracts disease only when subjected to definite preexisting conditions and that a predisposition or tendency to disease must exist.

Most plants inhabit two diverse environments since they are normally rooted in he soil but maintain light capturing organs and other structures in the atmosphere. Variables in either environment can profoundly affect the initiation and development of disease. Although weather and crops have been associated together in the minds of men for ages, it is only within the more recent times that there has been any real effort to determine the actual relationships.

The ability of a fungus to produce spores depends to a large extent upon the weather. Experiments and observations alike demonstrate that the abundance of a very large number of fungous diseases is directly connected with or conditioned by

climatological factors. Weather is the composite of the temperature, rain fall, light intensity and duration, wind direction and velocity, and relative humidity of a specific location over several years. It is the integrated effects of temperature, precipitation, humidity, sunlight, and wind.

Although all pathogens, all perennial, and, in warmer climates many annual plants are present in the field throughout the year, almost all diseases occur only, or develop best, during the warmer part of the year. Also, it is common knowledge that most diseases appear and develop best during wet, warm days, and that plants that are heavily fertilized with nitrogen usually are much more severely attacked by some pathogens than are less fertilized plants. These general examples clearly indicate that the environmental conditions prevailing in both air and soil, after contact of a pathogen with its host, may greatly affect the development of the disease, and frequently they determine whether a disease will occur.

The environmental factors that most seriously affect the initiation and development of infections plant diseases are temperature, moisture, light, soil nutrients, and soil pH.

It is obvious that, for a disease to occur and to develop optionally, a combination of three factors must be present : susceptible plant, effective pathogen, and favorable environment. Of course, a change in any environmental factor may favour the host, the pathogen, or both, or it may be more favourable to one than it is to the other, and the expression of disease will be affected accordingly. The extent and frequency of their occurrence, as well as the severity of the disease on individual plants, are influenced by the degree of deviation of each environmental condition from the point at which disease development is optimal.

The ability of the fungus to produce further infection will depend upon several factors, such as viability of the spores, susceptibility of the host plants, etc.

TEMPERATURE :

Temperature has been regarded as one of the most important variables affecting the development of biological system and its effects on the occurrence and development of many diseases have already been discussed by **Colhoun** (1973). Heat treatments prior to inoculation do not uniformly affect susceptibility of plants to infection by fungi. However, when viruses are concerned such pretreatment usually predisposes the plant to make it more susceptible.

Plants as well as pathogens require certain minimum temperatures in order to grow and carry out their activities. The low temperatures of late fall, winter, and early spring are below the minimum required by most pathogens. With the advent of higher temperatures, however, pathogens become active and, when other conditions are favourable, they can infect plants and cause disease. Pathogens differ in their preference for higher or lower temperatures; many diseases develop best in areas, seasons, or years with cooler temperatures, while others develop best

where and when relatively high temperatures prevail. Thus some species of the fungi Typhula and Fusarium, which cause snow mold of cereals and turf grasses, thrive only in cool seasons or cold regions. Also, the late blight pathogen Phytophthora infestans is most serious in the northern latitudes; in the subtropics it is serious only during the winter. More diseases are favoured by high temperature and limited in range to areas and reasons in which such temperatures are prevalent. Such diseases included the fusarial wilts of plants, the Phymatotrichum roots rots of plants, the brown rot of stone fruits caused by Monilinia fructicola, and the southern bacterial will of solanaceous plants caused by Pseudomonas solanacearum.

Numerous instances have been recorded in which susceptibility to disease has been altered by the temperature to which storage organs like fruits and potato tubers have been subjected before inoculation. **Vasudeva** (1930) established that by heating apples at 30^0C for 17 days they became susceptible to attack by Botrytis allii, whereas similar fruits held at the normal laboratory temperature prior to inoculation

remained immune to attack. Similarly, **Chona** (1932) should that maintaining apples at 35^0C for 1 week prior to inoculation rendered them liable to slight attack by Fusarium solani var. coeruleum (=F. caeruleum), to which they are not susceptible under normal conditions. Moreover, apples receiving this heat treatment were more susceptible when inoculated with F. lateritium (=F. fructigenum) than were those kept at 15^0C prior to inoculation.

The most rapid disease development, i.e., the shortest time required for the completion of a disease cycle, usually occurs when the temperature is optimum for the development of the pathogen but is about or below the optimum for the development of the host. Thus, for stem rust of wheat, caused by Puccinia graminis tritici, the time required for a disease cycle (from inoculation with uredospores to new uredospore formation) is 22 days at 5^0 C, 15 days at 10^0 c, and 5 to 6 days at 23^0 C. If the minimum, optimum and maximum temperatures for the pathogen, the host, and the disease are about the same, the effect of temperature in disease development is apparently

through its influence on the pathogen, which becomes so activated at the optimum temperature that the host, even at its optimum growth rate, cannot contain it.

In the black root rot of tobacco, caused by the fungus *Thielaviopsis basicola*, the optimum temperature range for disease is 17 to 23^0 C, and that for the pathogen is 22 to 28^{0}C. Evidently, neither the pathogen nor the host grow well at 17-23^{0}C, but the host grows so much more poorly and is so much weaker than the pathogen that even the weakened pathogen can cause maximum disease development. In the root rots of wheat and corn, caused by the fungus *Gibberella zeae*, the maximum disease development on wheat occurs at temperatures above the optimum for development of both the pathogen and wheat, but on corn it occurs at temperatures below the optima for the pathogen and corn.

The optimum temperature for growth of a particular fungus in culture may not be the optimum temperature for spore germination or for spore formation. In general, the optimum temperature for the

bacteria and fungi with which the pathologist is concerned would lie between 25^0 and 32^0C and it is customary to run an incubator in which ordinary cultures are being kept for vigorous growth and development at from 26^0 and 28^0C.

The thermal death points for vegetative cells of the bacteria and fungi have been variously determined to range from 40^0 to 75^0C. As a rule, few fungi will grow above 40^0, and to this temperature most of these organisms will after a time succumb .

In general, fungi are able to withstand very low temperatures. Few fungous spores are injured at 0^0C. The effects of winter conditions are not ordinarily such as to destroy fungous spores to any great extent.

Moisture : Moisture, like temperature, influences the initiation and development of infectious plant diseases, in many interrelated ways. It may exist as rain or irrigation water on the plant surface or around the roots, as relative humidity in the air, and as dew. Moisture seems to have the greatest

influence on the germination of fungal spores and on the penetration of the host by the germ tube. It also activates bacterial, fungal, and nematode pathogens, which may then infect the plant. Moisture, in such forms as splashing rain and running water, also plays an important role in the distribution and spread of many of these pathogens on the same plant or from one plant to another. Finally, moisture affects the extent and severity of disease by increasing the succulence of host plants and thus considerably increasing their susceptibility to certain pathogens.

The occurrence of many diseases in a particular region is closely correlated with the amount and distribution of rainfall within the year. Thus, late blight of potato, apple scab, downy mildew of grapes, and fire blight are found or are severe only in areas with high rainfall or high relative humidity during the growing season. In fungal diseases, the moisture has an effect on fungal spore formation and longevity, and particularly on the germination of the spores which require a film of water covering the tissues in order to germinate. In many fungi, moisture

also affects the liberation of spores from the sporophores which, as in apple scab, can occur only in the presence of moisture. In apple scab, for example, continuous wetting of the leaves, fruit, etc., for at least 9 hours is required for any infection. to take place even at the optimum range (18 to 23^{0}C) of temperature for the pathogen. At lower or higher temperatures the minimum wetting period required is higher, for example, 14 hours, at 10^{0}C, 28 hours at 6^{0}C..

Most fungal pathogens are dependent on the presence of free moisture on the host or high relative humidity in the atmosphere only during germination of their spores and become independent once they can obtain nutrients and water from the host. Some pathogens, however, such as those causing late blight of potato and the downy mildews, must have high relative humidity or free moisture in the environment though their development.

Though most fungal and bacterial pathogens of aboveground parts of plants require a film of weather

in order to infect hosts successfully, the spores of the powdery mildew of fungi can germinate, penetrate, and cause infection even when there is only high relative humidity in the atmosphere surrounding the plant. In the powdery mildews, spore germination and infection are actually lower in the presence of free moisture on the plant surface then they are in its absence and, in some of them, the most severe infections take place when the relative humidity is rather low (50 to 70 percent). Weather conditions may have substantial effects on both soil moisture and atmospheric humidity, but they may also predispose plants to disease in other ways. For example, it has been noted that during storms tobacco leaves often develop water-soaked areas due to flooding of the intercellular spaces. It has been suggested by **Colhoun** (1962) that high atmospheric humidity during the growing season induces greater resistance of lenticels on apple fruits to penetration by Penicillium expansum during their later storage life. Exposure of tomato plants to low soil moisture for 30 days prior to inoculation with Fusarium oxysporum f. sp. lycopersici

made plants more susceptible. (**Foster** and **Walker**, 1947). **Ghaffar** and **Erwin** (1969) concluded that water stress is more important than temperature in predisposing cotton plants to severe attacks of Macrophomina phaseolina. In the Pythium damping off of seedlings and seed decays - the severity of the disease is proportional to the amount of soil moisture and is greatest near the saturation point. Many other soil fungi (for example, Sclerotium), some bacteria and most nematodes usually cause their most sever symptoms on plants when the soil is wet but not flooded. Several other fungi, for example, Fusarium solani, which is the cause of dry root rot of beans, Fusarium roseum, the cause of seedling blights, and Macrophomina phaseoli, the cause of charcoal rot of sorghum, and of root rot of cotton grow fairly well in rather dry environments and apparently that enables them to cause more severe diseases in drier soils on plants that are stressed by insufficient water.

Many fungous diseases are directly associated with abundant precipitation, or a humid atmosphere. There is no more conspicuous example of this than the

brown rot of stone fruits - a disease which, in moist weather, has repeatedly crippled the peach industry. The association of epidemics of such diseases as black rot of the grape, apple scab, and late blight of the potato with humidity is a matter of almost annual record.

LIGHT : Light, darkness, or shading may be predisposing to attack by fungi or viruses.

The effect of light on disease development, especially under natural conditions, is far less than that of temperature or moisture, although several diseases are known in which the intensity and/or the duration of light may either increase or decrease the susceptibility of plants to infection and also the severity of the disease.

The chief role of light in plant economy is connected with the formation of sugar and starch, from which, in large part, the other organic products are ultimately derived. Light, however, calls forth a variety of responses in every green plant, and it may play a direct or indirect role in the relation with

parasitic fungi. In nature, however, the effect of light is limited to the production of more or less etiolated plants as a result of reduced light intensity. This usually increases the susceptibility of plants to nonobligate parasites, for example, of lettuce and tomate plants to Botrytis or of tomato to Fusarium, but decreases their susceptibility to obligate parasites, for example, of wheat to the stem rust fungus Puccinia.

Samuel and **Bold** (1933) established that shading tobacco plants for 3 days made them more susceptible than unshaded plants to the spotted wilt virus. **Bawden** and **Roberts** (1947,1948)proved very clearly that a number of hosts became more susceptible to viruses if kept in the dark for short periods before inoculation. The increase in susceptibility resulting from dark treatment appeared to be roughly proportional to the amount by which photosynthesis was reduced. **Wiltshire** (1956a,b) demonstrated that plants maintained for a time in the light but without carbon dioxide prior to inoculation became as susceptible as plants maintained in the dark. **Helms** and **Mc Intyre** (1967 a,b)showed that

bean plants exposed to light for 2-3 minutes after a dark period were more susceptible to tobacco mosaic virus than those without the light period.

Result obtained by **Coast** and **Chant** (1970) showed that plants exposed to blue or red light before inoculation with tobacco necrosis virus were less susceptible than those exposed to green light. These results support the hypothesis of **Bawden** and **Roberts** (1948) that susceptibility is related to photosynthetic activity because chlorophyll a and b absorb more light energy of the red and blue wavelengths than of the green wavelengths. **Brooks** (1908) found that spores of Botrytis cinerea in drops of water did not attack leaves of normal green lettuce plants, but if the leaves were kept in the dark for 5 days they were readily invaded. **Horsfall** and **Dimond** (1957) attempted to explain the effects of dark treatments on the susceptibility of plants to fungi in terms of sugar being used up by respiration in the dark so that such plants become more susceptible to low-sugar diseases such as those caused by Fusarium sp.

Reduced light intensity generally increases the susceptibility of plants to virus infections. Holding plants in the dark for one to two days before inoculation increases the number of lesions (that is, infections) appearing after inoculation. On the other hand, low light intensities following inoculation tend to mask the symptoms of some diseases, which are much more severe when the plants are grown in normal light than when they are shaded.

It has been observed that celery under lath or cloth screens, that is, half shade, is largely free from the early blight. In opposition to this beneficial effect of half shade, there are also abundant observations showing that certain powdery mildews are far more effective as parasites under just such conditions as above enumerated. There are instances where wheat under partial shade is seen to be badly infested with the powdery mildew, which is seldom seen in the open.

It is a matter of common observation that while cucumbers frequently mildew under greenhouse

conditions, yet in the open the cucumber mildew is very seldom observed upon this host. It is claimed that certain greenhouse plants are more subject to the attack of the common gray mold, Botrytis, when partially etiolated and **De Bary**, it seems, was able to predispose Petunia to the attack of Botrytis through etiolation.

There may also result a number of direct effects of light, for in the case of strongly etiolated or yellowed and attenuated plants, bud and stem diseases seem frequently to be more common. Very little experimental study has been bestowed upon these relations.

Soil pH :

Soil may harbour many important plant parasites which can be devastating if attempts are made to grow susceptible crops. They include parasitic angiosperms, such as Striga and Orobanche, nematodes and fungi; their resting structures, i.e. seeds, cysts and spores, respectively, may survive in the soil, for

many years making the economic cultivation of important crops difficult or impossible.

Soils vary considerably in many factors that affect disease development - moisture content, temperature and pH being among the most important. In some instances the overriding influence of these factors is on the parasite while in others it is on the host. In addition, soils normally harbour a microbial flora, some members of which may be antagonistic to plant parasites.

The pH of the soil is important in the occurrence and severity of plant diseases caused by certain soil-borne pathogens. For example, the clubroot of crucifers, caused by Plasmodiophora brassicae, is most prevalent and severe at about pH 5.7, while its development drops sharply between 5.7 and 6.3, and is completely checked at pH 7.8. On the other hand, the common scab of potato, caused by Streptomyces scabies, can be severe at a pH range from 5.2 to 8.0 or above, but its development drops sharply below pH 5.2. It is obvious that such diseases are

most serious in areas whose soil pH favours the particular pathogen.

Low pH can have suppressive effects on plant disease. For example, sporangium formation, zoospore release and motility of zoospores of *Phytophthora cinnamoni* was reduced by pH values less than 4.0 (**Blaker** and **Mc Donald**, 1983). On the other hand raising the pH by liming has long been used to mitigate clubroot of brassicae caused by *Plasmodiophora brassicae*.

In addition, there are organisms in the soil that are antagonistic to plant pathogens. For example, over 100 species of fungi trap and prey on nematodes (**Jatala**, 1968) and many fungi are hyperparasites of other fungi (**Adanis**, 1990). Among these are the species of *Trichoderma* that secrete lytic enzymes which are active against fungal cell walls (**Sivan** and **Chet**, 1989), *Talaromyces flavus* which is able to attack the sclerotia of *Sclerotinia slerotiorum* and *Verticillium* which is able to attack the sclerotia of five important plant pathogens (**Adams**, 1990).

NUTRIENTS : Nutrition affects the rate of growth and the state of readiness of plants to defend themselves against pathogenic attack.

The case with which fungi may be grown in cultures and the use of synthesized culture media have afforded an opportunity for exact determination of the elements required by these organisms. There may be some specific variations, but it is now generally agreed that the majority of the fungi require nine elements, viz., carbon, hydrogen, oxygen, nitrogen, sulphur, phosphorus, potassium, magnesium, and iron.

It has been assumed that when manurial treatments of plants are associated with changes in susceptibility, these are due to changes in the host. Thus, **Moore** (1944) considered that deficiency of potassium or available phosphate in soil predisposes beans to attack by Botrytis fabae.

Few experiments have been made in which the susceptibility of plants has been clearly shown to be altered by the nutrition of plants prior to inoculation. However, **Foster** and **Walker**, (1947) found

that the susceptibility of tomatoes to Fusarium oxysporum f. sp. lycopersici is increased if the nutrient they receive for 30 days prior to inoculation is low in nitrogen or phosphorus or high in potassium. In experiments on the root rot of chrysanthemums caused by Phoma chrysanthemicola, it has been established that heavy applications of fertilizers, particularly nitrogen and phosphate, gives good control (**Peerally** and **Colhoun**, 1969; **Menzies** and **Colhoun**, 1976 a,b).

For most culturable fungi, whether primarily parasitic or saprophytic, carbon is available as grape or cane sugar, glycerine, asparagin, peptone, etc., in fact, in almost any soluble or readily convertible nontoxic form. It is to be inferred that the obligate parasite, as well, utilizes the soluble carbohydrates, peptones, etc., of the host cell, but its exact relations cannot well be determined.

Nitrogen may be furnished to the readily culturable fungi in the form of nitrates or ammonia compounds, but in some cases preferably as peptone,

casein, or in other organic form. Nitrogen abundance results in the production of young, succulent growth and may prolong the vegetative period and delay maturity of the plant. These effects make the plant more susceptible to pathogens that normally attack such tissues, and for longer periods. Conversely, plants suffering from a lack of nitrogen would be weaker, slower growing, and faster aging and susceptible to pathogens that are best able to attack weak, slow growing plants. Reduced availability of nitrogen may increase the susceptibility of tomato to *Fusaruim* wilt, of many solanaceous plants to *Alternaria solani* early blight and *Pseudomonas solanacearum* wilt, of sugar beets to *Sclerotium rolfsii*, and of most seedlings to *Pythium* damping off.

Of numerous root rots, wilts, foliar diseases, etc., treated with either form of nitrogen, almost as many decreased or increased in severity when treated with a source of ammonium nitrogen as did when treated with a source of nitrate nitrogen, but each form of nitrogen had exactly the opposite effect on a disease (that is, decrease or increase its severity) than did

the other form of nitrogen. For example, Fusarium spp., Plasmodiophora brassicae, Sclerotium rolfsil, and the diseases they cause (root rots and wilts, clubroot of crucifers, and damping off and stem rots, respectively) increase in severity when an ammonium fertilizer is applied, while Phymatotrichum omnivorum, Gaeumannomyces graminis, Streptomyces scabies and the diseases they cause (cotton root rot, take all of wheat, and scab of potato, respectively) are favoured by nitrate nitrogen.

Phosphorus has been shown to reduce the severity of take all disease of barley (caused by Gaeumannomyces graminis) and potato scab (caused by Streptomyces scabies), but to increase the severity of cucumber mosaic virus on spinach and glume blotch (caused by Septoria) on wheat.

Potassium has also been shown to reduce the severity of numerous diseases, including stem rust of wheat, early blight of tomato, and stalk rot of corn, although high amounts of potassium seems to increase the severity of rice blast (caused by Pyricularia

oryzae) and root knot (caused by the nematode Melioidogyne incognita). Potassium seems to directly affect the various stages of pathogen establishment and development in the host, and to indirectly affect infection by promoting wound healing by increasing resistance to frost injury and thereby reducing infection that commonly begins in frost-killed tissues, and by delaying maturity and senescence in some crops beyond the periods in which infection by certain facultative parasites can be severely damaging.

Reduction in disease levels was also observed when the levels of certain micronutrients were increased. For example, applications of iron to the soil reduced Verticillium wilts of mango and of peanuts, and foliar applications of iron compounds reduced the severity of silver leaf of deciduous fruit trees (caused by Sterium purpureum). Similarly, applications of manganese reduced potato scab and late blight of potato and stem rot (caused by Sclerotinia sclerotiorum) of pumpkin seedlings, while applications of molybdenum reduced late blight of potato and

Ascochyta blight of beans and peas. However, even a balanced nutrition may affect the development of a disease when the concentration of all the nutrients is increased or decreased beyond a certain range.

WIND: Wind influences infections plant diseases primarily through its importance in the spread of plant pathogens and, to a smaller extent, through its acceleration of the drying of wet plant surfaces. Some spores, for example, zoosporangia, basidiospores, and some conidia, are quite delicate and do not survive long distance transport in the wind. Others, for example, uredospores and many kinds of conidia, can be transported by the wind for many miles. Wind is even more important in disease development when it is accompanied by rain. Wind blown rain helps release spores and bacteria from infected tissue and then carries them through the air and deposits them on wet surfaces which, if susceptible, can be infected immediately. Wind also injures plant surfaces while they are blown about and rub against each other; this facilitates infection by many fungi and bacteria and also by some mechanically transmitted viruses. Wind,

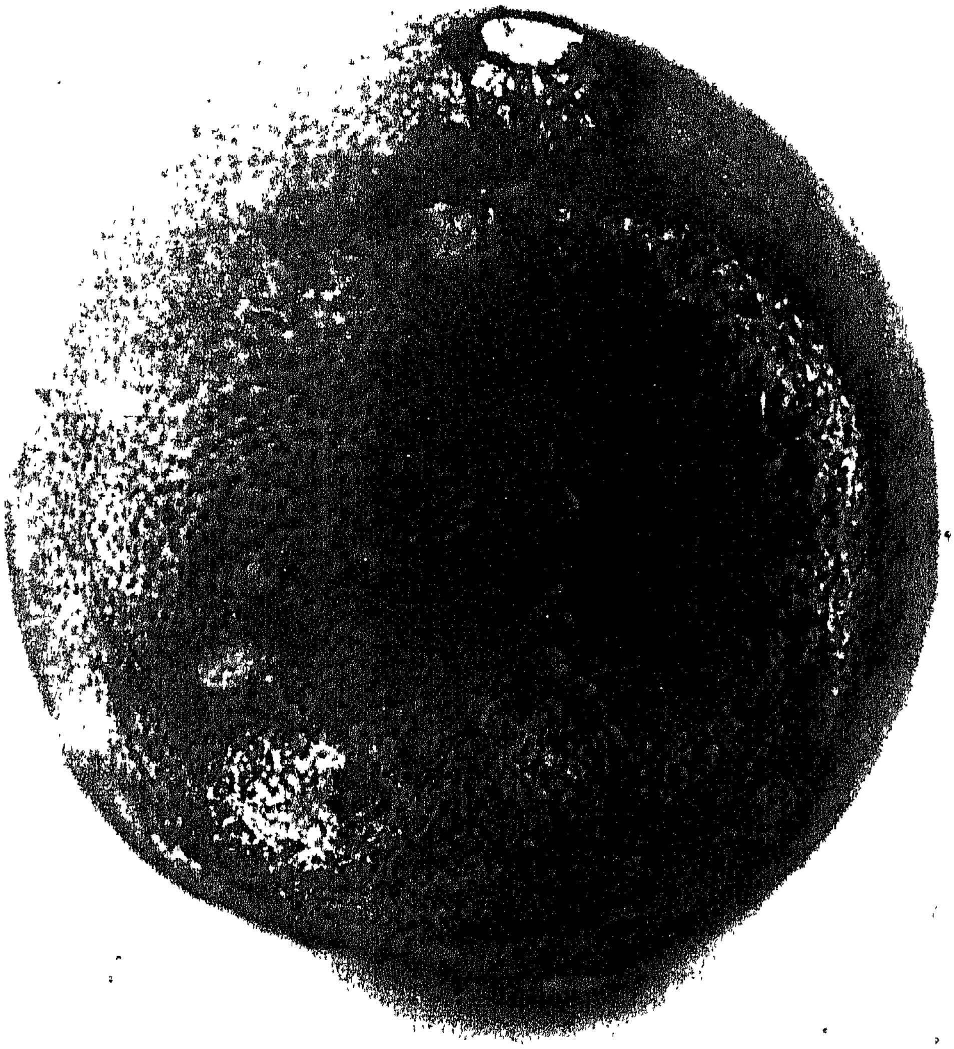
however, sometimes helps prevent infection by accelerating the drying of the wet plant surfaces on which fungal spores or bacteria may have landed. If the plant surfaces dry before penetration has taken place, any germinating spores or bacteria present on the plant are likely to desiccate and die and no infection will occur.

HERBICIDES: Herbicide use is common and widespread in agriculture. In many cases, herbicides have been shown to increase the severity of certain diseases on crop plants, for example, of Rhizoctonia solani on sugar beets and cotton, Fusarium wilt of tomatoes and cotton, and Sclerotium stem rots of various crops. In other host pathogen combinations, herbicides appear to decrease disease, for example, Aphanomyces euteiches root rot of peas, Pseudocercosporella herpotrichoides foot rot of wheat , and Phytophthora collar rot of various crops. Herbicides apparently act on plant diseases either directly by affecting (stimulating or retarding) the growth of the pathogen or increasing or decreasing the susceptibility of the host, or indirectly by increasing or decreasing the activity of

soil microflora, by eliminating or selecting for certain additional or alternate hosts of the pathogen, or by altering the microclimate (for example, humidity) of the crop plant canopy.

It has long realized that plant diseases are greatly influenced by the environment Theophrastus (370-286 B.C.) believed that the position and character of the land made a considerable difference with respect to attack of rusts on cereals. He believed that crops growing on elevated land exposed to the wind were not liable to rust, or were less so than those growing on lowlands not exposed to wind. It was accepted at that time that microorganisms associated with diseased plants arose spontaneously from the plants or possibly form the environment. This theory of spontaneous generation dominated the thoughts of those studying plant diseases for more than 2000 years.

LOSSES



"The difference between those who advance the frontiers and those who merely exist in science is the ability to choose the right problem."

[E.L. ELLWOOD]

The basic position of green plants in the maintenance of life on this planet should never be forgotten in any consideration of plant problems. With exceptions which are surely negligible in this discussions green plants alone are capable of making available to other living organisms the energy of the sun - the ultimate source of virtually all energy which can be so made available. On green plants also other living things are dependent or their supply of oxygen. So with food, not only the carbohydrates but most of the amino acids, even some fatty acids, and many of the vitamins must be synthesized in green plants before they can be used by organisms lacking chlorophyll.

"The history of mankind is the story of a hungry creature in search of food," wrote historian **Hendrick Van Loon.** The need for food has always governed the movement and activities of human beings. The welfare of plants is of particular interest to those most directly concerned with the growth of plants and the manufacture and distribution of plant products. Such persons include not only farmers and workers, in industries that process agricultural products but also immarable workers in supporting industries whose livelihood depends on making equipments or products used in processing plant products. Most importantly, however, the welfare of plants should be of concern to every one of us as growers of plants for food or pleasure, as individuals concerned with the beauty and safety of our natural environment, and particularly, as consumers of plants and of the endless series of products derived from plants.

A plant is healthy, or normal, when it can carryout its physiological functions to the best of its genetic potential. These functions include normal

cell division, absorption and translocation of substances, photosynthesis, etc. Whenever plants are disturbed by pathogens or by certain environmental conditions and one or more of these functions is interfered with beyond a certain deviation from the normal, then the plants become diseased. The ability of cells and tissues of diseased plants is reduced or completely eliminated; as a result, plant growth is reduced or the plant dies. **Zadoks** (1985) gives a fuller discussion of the economic and sociological effect of plant disease.

Plant diseases are significant to humans because they cause damage to plants and plant products. For millions of people all over the world who still depend on their own plant produce for their existence, plant diseases can make the difference between a happy life and a life haunted by hunger or even death from starvation. The death from starvation of a quarter million Irish people in 1845 and much of the hunger of the underfed millions living in the underdeveloped countries today are examples of the consequences of plant diseases. For countries where

food is plentiful, plant diseases are significant because they cause economic losses to growers, they result in increased prices of products to consumers, and they destroy the beauty of the environment by damaging plants around homes, along streets, in parks, and in forests.

Plant diseases may limit the kinds of plants that can grow in a large geographic area by destroying all plants of certain species that are extremely susceptible to a particular disease. Plant diseases may also determine the kinds of agricultural industries and the level of employment in an area by affecting the amount and kind of produce available for local canning or processing. On the other hand, plant diseases are responsible also for the creation of new industries that develop chemicals, machinery, and methods to control plant diseases.

The kinds and amounts of losses caused by plant diseases vary with the plant or plant product, the pathogen, the locality, the environment, the control measures practiced, and combinations of these factors.

The quantity of loss may range from slight to 100 percent loss. Plants or plant products may be reduced in quantity by disease in the field, or by disease during storage. Frequently, severe losses may be incurred by reduction in the quality of plant, products.

Plant diseases may cause financial losses in the following ways: Farmers may have to plant varieties or species of plants that are resistant to disease but are less productive, more costly, or commercially less profitable than other varieties. They may have to spray or otherwise control a disease, thus incurring expenses for chemicals, machinery, storage space, and labour. Shippers may have to provide refrigerated warehouses and transportation vehicles, thereby increasing expenses. Plant diseases may limit the time during which products can be kept fresh and healthy, thus forcing growers to sell during a short period of time when products are abundant and prices are low. Healthy and diseased plant products may need to be separated from each other, thus increasing handling costs.

Some plants diseases can be controlled almost on entirely by one or another method, thus resulting in financial losses only to the amount of the control. Sometimes, however, this cost may be almost as high as, or even higher than, the return expected form the crop, as in the case of certain diseases of small grains. For most plant diseases, however practical controls are available, although some losses may be incurred in spite of the control measures taken.

The paucity of literature on the economic aspects of disease control may be a reflection of the intrinsic difficulty of the subject. The most casual reading of a "check list" of known plant pathogens makes it clear that most of them have never been the object of serious attempts at control. From the viewpoint of growers of crops, and for that matter of consumers, it is a matter of no small importance to determine which diseases are worth an attempt at control and under what circumstances active control measures may be undertaken with a reasonable assurance of adequate returns. A reasonable answer to a question as to which diseases are of greatest economic

importance will depend on the area considered. Apparently the problem has not yet been attacked on a world basis. Recently, however, **Mc Callan** has published a challenging summary for the United states of America. In his paper (I) available information regarding the value of various crops is combined with estimates of crop losses to give a single "disease importance index".

At this particular juncture in man's history, the importance of food and of preventable losses in food is receiving national and international attention at high administrative levels. During recent years, moreover, there has come increasing recognition of the fact that even when many countries suffered from what were regarded as dangerous surpluses of agricultural products, large sections of the population were not being adequately fed.

Press releases (12) regarding the London 1947 conference of the food and Agricultural Organisation of the United Nations stressed the fact that not only must the world produce more food, it must save what it

produces. The official statement read, in part: "under adverse climatic and unsatisfactory storage conditions the annual loss of grains, pulses [peas, beans, etc.], and oil seeds through infestation may run much higher than ten percent in some countries..... A 5 percent world average would represent about one-half the amount of these foodstuffs entering world trade." Naturally these estimates related to losses from insects as well as those from plant disease.

Any discussion of the importance of plant diseases at once raises the question of crop loss estimates and their validity. The published estimates of losses due to plant diseases have been subjected to repeated criticism. Whether we admit it or not disease loss estimates from an important part of the background of our professional activities. Of first importance are informed estimates regarding national losses in important crops. Estimates made prior to the last war for European countries vary form 25% to approximately 10%.

Most phases of plant pathology are of vital concern to consumers. Inevitably, consumers as a group bear practically all the cost of losses from plant diseases and derive the larger portion of the benefit of disease control. "Market" pathology as a somewhat specialized field is of particular interest to a special group of consumers; namely those who live a significant distance from the areas where their food is produced. The more remote either in time or space the area of consumption of fresh fruit and vegetables is from the area of production, the more important are diseases which cause losses between producer and consumer. Diseases occurring on fresh fruits and vegetables while in transit, in storage, and on the market and the means of controlling these diseases constitute the special field of market pathology.

Population trends and dietary changes alike have greatly increased the importance of transportation and storage of fruits and vegetables. Adequate and extremely useful summaries of our knowledge of the causes and means of prevention of storage losses after harvest together with numerous

references to literature are found in an article by **C.W. Wardlaw** (?).

Accurate information regarding losses due to disease is easier to obtain when the wastage occurs after the crop is harvested. Statements regarding crop productions due to plant disease are of necessity based largely on estimates. In the case of losses occurring after the crop is stored or shipped more satisfactory measurements of different kinds may be made. For example, during the 10 year period from 1921-1931, American railroads paid claims for "loss and damage" on fruits and vegetables ranging from slightly over $ 9,000,000 to almost $ 14,000,000 each year. **Brooks** gives figures for the average claims figures for the average claims per car paid by the American railroads in 1932. He notes that the claims paid on eggs, certainly a not too solid product, averaged $ 3.95 on a car and for glass $ 1.07 a car. In other words, the loss on tomatoes was more than six times that on eggs, that on sweet potatoes three times the loss on glass.

Relatively accurate information on extent of losses during transit is available from the reports of the Foods Products Inspection Service of the United States Department of Agriculture for two highly perishable crops, strawberries and tomatoes. During the period 1919 to 1925 68 percent of all the cars of strawberries inspected showed some decay. During the years 1926 to 1930, the member showing decay was slightly under 60 percent. In the earlier period the percentage decay was 8.2, for the 5 years, 1926 to 1930 the average was about 4 percent. During the year 1930 an average of $46-04 for damage per car was paid by the American railroads for all the tomatoes they carried, as compared with an average for all commodities of $ 11.25 per car.

The growing world food problem cannot possibly be adequately addressed in one single chapter, but it is possible to consider some important aspects of this challenge that directly relate to the science of plant pathology. The world population is increasing, so food supplies must also increase. In some areas of the world, particularly in Africa, Mexico, and parts of

Asia, growth rates are more than 3.5%. The world population, which now exceeds 5 billion, reached its first billion around year 1800. The population doubled by 1930, and doubled once more by 1975. Even with extensive food shipments and economic aid, it is hard to imagine the technological, political, and sociological problems in providing for a population that increases so quickly. Paradoxically, the developing countries, in which 57.6 percent of the population is engaged in agriculture, have the lowest agricultural output, their people are living on a substandard diet, and they have the highest population growth rates (2.64 percent). Because of the current distribution of usable land and population, educational and technical levels for food production and of general world economics, it is estimated that even today some 800 million people are undernourished and 2.0 billion suffer from hunger or malnutrition or both. To feed these people and the additional millions to come in the next few years, all possible methods of increasing the world food supply are currently being pursued, including: (1) expansion of crop acreages,

(2) improved methods of cultivation, (3) increased fertilization, (4) use of improved varieties of crops, (5) increased irrigation, and (6) improved crop protection.

Crop losses to diseases and pests not only affect national and world food supplies and economics but affect even more individual farmers, whether they grow the crop for direct consumption or for sale. Since operating expenditures for the production of the crop remain the same, harvest lost to disease and pests directly lower the net return.

In the final analysis severity of disease is not the same as loss, and its with the latter that mankind is really concerned. If diseased plants produced the same amount of food and fiber, of the same quality, as did healthy plants we would be for the most part unconcerned about them. But measuring disease severity and translating it into measurements of loss is a rather complex question. For the outset they may be considered separately.

CHANGE IN NUTRIENTS

Fungi differ from plants most conspicuously in lacking photosynthesis. They are heterotrophs dependent on external supply of readymade organic food, which they procure as parasites or saprobes. Unlike animals, which are also heterotrophs, their nutrition is absorptive; ingestion is rare and restricted only to the slime molds. The hyphae lie in direct contact with the nutrients and absorb dissolved smaller molecules such as simple sugars and amino acids. Large insoluble substances like polysaccharides, fats, proteins, etc. - are first broken into smaller fragments until the soluble monomers are released. Fungi are extremely versatile in their nutrition. Except certain fluorine-containing plastics and a few detergents, there is nothing which fungi cannot degrade to derive nutrition.

Fungi causing rots of vegetables and fruits generally depend for these nutrition on the stored products of the host tissues. Thus the association of fungi with the diseased fruit and vegetables have

several effects on the stored product or on the nutrients value of the host. As a result of their metabolic activities changes are brought about inside the host tissues. The process of change in the nutrient value of the host is quite complicated. But to study the behaviour of the pathogens, it is necessary to study the changes in amino acids, organic acids, sugars and ascorbic acid of the host tissue. So far not much is done to study the changes brought about in the nutrient contents of Pineapple, Snake gourd and Carambola infected by Alternaria alternata, Fusarium oxysporum and Botryodiplodia theobromae respectively.

To study such changes these fruits were inoculated with the pure cultures of their specific isolated of their respective pathogens and the analysis of the infected as well as the healthy tissue was carried out at the intervals given in the tables. Methods of preparation of ethanolic extracts for the analysis of amino acids, organic acids, and sugar have been described in chapter II dealing with materials

and methods. These substances were detected with the help of paper chromatographic techniques.

(1) **<u>Post infection changes in amino acids</u>** : Several workers including **Tandon** (1970), **Jamaluddin** (1973), **Arjunan** <u>et</u> <u>al</u>. (1976), **Thind** <u>et</u> <u>al</u>. (1977), **Singh** and **Chohan** (1977), **Rai** (1982), **Arya** (1982), **Pandey** (1983), **Tiwari** (1986), **Agarwal** (1987), and **Malviya** (1992) have studied the post infection changes in the amino acids are the main source of nitrogen for growth and sporulation of the pathogen in their hosts. They are either present as such or in protein complex.

In the present investigation, qualitative analysis of amino acids of healthy as well as diseased fruits & vegetables were carried out after 12 days of incubation. The results are summarized in table-5.

<u>Pineapple</u> : From the results of table-5, it is concluded that the fruit of pineapple contained leucine/isoleucine, valine, γ-amino butyric acid, β-alanine, glutamic acid, aspartic acid, serine/glycine, asparagine, glutamine, histidine/ lysine and an unidentified amino acid in the free form.

Table-5:- Free and bound amino acids in ethanolic extracts of healthy and diseased fruits of pineapple. infected with <u>A</u>. <u>alternata</u>.

Amino acids	Free amino acids		Bound Amino acids	
	Healthy	Diseased	Healthy	Diseased
Leucine/isoleucine	+	T	2+	+
Valine	+	-	-	-
γ-amino butyric acid	+	-	-	-
β-alanine	+	2+	+	2+
Glutamic acid	2+	+	T	-
Threonine	-	-	2+	+
Aspartic acid	+	+	+	+
Serine/glycine	4+	2+	+	+
Asparagine	3+	+	-	-
Glutamine	2+	+	+	+
Histidine/lysine	2+	+	+	-
Cystine	-	-	+	T
Unidentified	+	T	-	-

T = Trace

Due to the infection caused by A. alternata, valine and γ-amino butyric acid are completely exhausted. Also, leucine/isoleucine and an unidentified amino acid were detected in traces. The amount of glutamic acid, Serine/glycine, asparagine, glutamine and histadine/lysine decreased while there is an increased concentration of β-alanine. The concentration of aspartic acid remained unchanged.

In bound state leucine/isoleucine, β-alanine, glutamic acid, threonine, aspartic acid, serine/glycine, glutamine, histidine/lysine, cystine are present. After infection with <u>A. atternata</u> <u>cystine</u> was detected in traces, while the concentration of leucine/isoleucine and threonine were decreased. The concentration of aspartic acid, serine/glycine and glutamine remained unchanged, while there is an increased concentration of β-alanine during pathogenesis.

<u>Snake gourd</u> : The results from the table-6, 'indicate that free amino acids present in the healthy snake gourd consists of leucine / isoleucine, valine,

Table-6:- Free and bound amino acids in ethanolic extracts of healthy and diseased fruits of snake gourd infected with F. *oxysporum*.

Amino acids	Free amino acids		Bound Amino acids	
	Healthy	Diseased	Healthy	Diseased
Leucine/isoleucine	+	+	-	+
Valine	2+	+	T	+
γ-aminobutyric acid	+	2+	T	2+
β-alanine	2+	+	T	+
Glutamic acid	+	+	+	-
Threonine	-	T	+	+
Aspartic acid	2+	2+	+	+
Serine/glycine	2+	+	4+	2+
Asparagine	+	+	+	-
Glutamine	+	+	+	2+
Histidine/Lysine	+	+	-	T
Cystine	+	T	+	+
Unidentified	-	-	T	T

T = Trace

γ-aminobutyric acid, β-alanine, glutamic acid, aspartic acid, serine/glycine, asparagine, glutamine, histidine/lysine and cystine. There is a decreased concentration of valine, β-alanine, serine/glycine, after the infection of of F. oxysporum. On the other hand there is an increase in the concentration of γ-aminobutyric acid during pathogenesis. The concentration of leucine/isoleucine, glutamic acid, aspartic acid, asparagine, glutamine, histidine/lysine remains unchanged. Cystine and threonine was found in traces.

Bound amino acids of snake gourd included valine, γ-aminobutyric acid, β-alanine, glutamic acid, threonine, aspartic acid, serine/glycine, asparagine, glutamine, cystine and an unidentified amino acid. There was reduction in the concentration of asparagine and glutamic acid whereas there was an increase in the concentration of valine, γ-aminobutyric acid, β-alanine, and glutamine. The concentration of threonine, aspartic acid and cystine remains the same, while leucine/isoleucine and histidine/lysine appeared as a result of infection.

Table-7 :- Free and bound amino acids in ethanolic extracts of healthy and diseased fruits of Carambola infected with B. theobromae.

Amino acids	Free amino acids		Bound Amino acids	
	Healthy	Diseased	Healthy	Diseased
Leucine/isoleucine	+	T	-	+
Valine	+	2+	T	+
γ-aminobutyric acid	-	-	T	2+
Proline	-	-	-	+
β-alanine	2+	+	+	2+
Glutamic acid	+	+	+	+
Threonine	-	-	-	+
Aspartic acid	2+	+	2+	2+
Serine/glycine	2+	+	2+	+
Asparagine	+	-	-	-
Glutamine	2+	+	-	-
Histidine/Lysine	+	T	+	2+
Cystine	+	+	+	T
Unidentified	-	-	T	T

T = Trace

Carambola : From the table -7, it is concluded that free amino acid pool of healthy fruits consists of leucine/isoleucine, valine, β-alanine, glutamic acid, aspartic acid, serine/glycine, asparagine, glutamine, histidine/lysine and cystine. Due to the infection of B. theobromae, asparagine was completely lost. During pathogenesis, there was an increase in the concentration of valine but leucine/isoleucine, β-alanine, aspartic acid, serine/glycine, glutamine and histidine/lysine shows the reduction in their concentration. The concentration of cystine and glutamic acid remain unchanged.

In bound state, valine, γ-butyric acid, β-alanine, glutamic acid, aspartic acid, serine/glycine, hystidine/lysine, Cystine and unidentified amino acid are present in the healthy fruit of carambola. The concentration of valine, γ-aminobutyric acid, β-alanine, and histidine/lysine increased, while there was reduction in the concentration of cystine and serine/glycine. The concentration of glutamic acid, aspartic acid and an unidentified amino acid remains the same, while

leucine/isoleucine, proline and threonine appeared in the diseased fruit.

(2) <u>Post-infection changes in Organic acids</u> : **Ulrich** (1970) has critically reviewed the biochemistry of fruits in relation of organic acids. A number of investigations were carried out including the work of **Kapoor** and **Tandon** (1969a), **Bhargava** and **Tandon** (1976), **Thind** <u>et al.</u> (1977) and **Tewari** (1986). They studied the changes in the organic acid contents of certain fruits during the pathogenesis.

Organic acids play an important role in the physiological functions of plants, particularly in respiration, photosynthesis, synthesis of phenolic compounds and lipids. Hence, an attempt has been made to observe the changes in organic acid contents of different hosts during the pathogenesis. For this purpose 12 days old infected tissues were analysed chromatographically and the results are given in table-8.

From the table -8, the followings results were concluded-

Table-8:- Post infection changes in organic acids (mg/g fresh wt.) in Pineapple, Snake gourd and Carambola infected by A. alternata, F. oxysporum and B. theobromae respectively.

Organic Acid								
Fruit/ Vegetable	Fumaric	Succinic	Malonic	Malic	Citric	Tartaric	Oxalic	Total
Pineapple								
Healthy	-	-	0.620	0.479	1.932	0.258	-	3.289
Infected	-	0.310	0.431	0.121	1.960	0.120	-	2.942
Snake gourd								
Healthy	-	0.388	0.165	0.466	-	-	0.975	1.994
Infected	-	0.156	0.145	0.489	-	T	0.646	1.436
Carambola								
Healthy	-	0.210	-	1.685	2.210	-	-	4.105
Infected	-	0.160	T	1.420	2.110	0.112	0.129	3.931

The healthy fruit of pineapple contained malonic, malic, citric and tartaric acid. During the pathogenesis, there is a decrease in the quantity of malonic acid, malic acid - and tartaric acid, while the amount of citric acid is increased. During pathogenesis there is an addition of succinic acid.

The healthy fruit of Snake gourd consisted of succinic acid, malonic acid, malic acid and oxalic acid. After infection with F. oxysporum, the diseased fruit showed an increase in the quantity of malic and tartaric acid, whereas the quantity of succinic acid, malonic acid and oxalic acids were decreased.

Likewise, the healthy fruits of Carambola contains succinic acid, malic acid, and citric acid. The infected fruit of carambola have malonic-acid, tartaric acid and oxalic acid. Besides the organic acids mentioned above in the healthy fruit, there was an increase in the quantity of malonic acid, tartaric acid and oxalic acid, while a decrease in the quantity of succinic acid, malic acid and citric acid in the diseased fruit was noted.

It is clear from the above observations that during pathogenesis certain organic acids appeared. This may be due to the result of interaction between host and the pathogen. Also, it was found that certain organic acids were decreased in quantity which may be due to their utilization by the pathogens.

(3) <u>**Post-Infection Changes in Sugars**</u> : Changes in the sugar contents of diseased fruits by various fungal pathogens have been studied by many workers including **Singh** and **Tandon** (1970), **Chahal** and **Grover** (1972), **Grewal** and **Grover** (1974), **Sharma** and **Wahab** (1975), **Bhargava** and **Tandon** (1976), **Singh** and **Chauhan** (1977), **Rai** (1982), **Tewari** (1986) and **Malviya** (1992).

Sugars or carbohydrates are the chief source of energy. Sucrose, glucose, fructose, etc. are some of the important sugars normally present in many fruits. The most interesting feature of pathogenesis is the breakdown of the carbohydrates of the host tissues. Rotting of fruits is mostly accomplished by the utilization of carbohydrates present in them.

In order to find out the changes in sugar contents of healthy fruits after infection, they were analysed chromatographically after 4, 8, and 12 days of incubation period. The intensities of spots of a single sugar were visually compared with another and various concentrations were graded as +, 2+, 3+ and 4+. The sign '-' shows the absence of sugars. The observation is given in table -9 .

The healthy fruit of Pineapple showed the presence of the three sugars, namely' glucose, fructose and sucrose. These sugars were present upto end of incubation period but their concentration slightly decreased in the end. The inoculated fruits of pineapple showed the presence of glucose, fructose and sucrose in the beginning, after which they show the decrease in the concentration and upto the 12th day glucose was completely exhausted.

Healthy fruit of snake gourd contained glucose, fructose and sucrose. There was a slight loss of tnese sugars at the end of the incubation period. During pathogenesis glucose was lost on the 12th day,

Table-9:- Changes in Sugar contents of pineapple, snake gourd and carambola after infection.

Fruits/ Vegetable	Causal Organism	Sugars	Uninoculated				Inoculated			
			0	4	8	12	0	4	8	12
Pineapple	*A. alternata*	Glucose	4+	4+	3+	3+	4+	3+	+	-
		Fructose	3+	3+	2+	2+	3+	2+	+	+
		Sucrose	2+	2+	2+	+	2+	2+	+	+
Snake gourd	*F. oxysporum*	Glucose	2+	2+	2+	+	2+	2+	+	-
		Fructose	2+	2+	2+	+	2+	+	-	-
		Sucrose	2+	+	+	+	+	+	-	-
Carambola	*B. theobromae*	Glucose	4+	4+	3+	3+	4+	2+	+	-
		Fructose	3+	3+	2+	2+	3+	2+	+	-
		Sucrose	2+	2+	2+	+	2+	+	+	+

while sucrose and fructose was lost on the 8th day of incubation.

In the healthy fruits of carambola, glucose, fructose and sucrose are present. In healthy tissues there was slight decrease in the amount of sugar contents. During pathogenesis, glucose and fructose were lost on the 12th day of incubation, whereas sucrose was present upto the end of incubation period.

It is evident from the above observations that there is a pronounced loss of sugars in the diseased fruit as compared to healthy ones. These sugars may disappear slowly or reduced gradually.

(4) Post Infection Changes in the Ascorbic Acid Contents : The quantity of ascorbic acid (Vitamin C) decreases during the storage of healthy fruits. **Ghosh** *et al*. (1966), **Srivastava** and **Tandon** (1966b), **Singh** (1968), **Tandon** (1970), **Pandey** (1983), **Tewari** (1986) and **Malviya** (1992) have showed that fungal decay causes huge loss in ascorbic acid content of healthy and diseased fruits. Fruits are the chief source of Vitamin C and has great medicinal and curative value.

Its biosynthesis in plants have been extensively reviewed by **Mapson** (1958) and **Isherwood** and **Mapson** (1962).

It was, therefore, considered desirable to undertake a study of the loss of ascorbic acid content of healthy as well as diseased fruits after 4,8 and 12 days of incubation periods. The results are summarized in table —10.

It is clear from the observation table that with the increase in the incubation period, there was a gradual decline in the ascorbic acid content of healthy and infected fruit of pineapple, snake gourd and carambola. But, it was noted that this loss is comparatively faster in the diseased fruits than healthy one.

The percentage loss of ascorbic acid in pineapple infected with <u>A</u>. alternate was 80.4%, while that in healthy fruit was only 51.2%. In case of Snake gourd infected with <u>F</u>. <u>oxysporum</u>, this loss was 82.8%. but in the healthy fruit was 55.8%. Likewise, the

Table-10:- Changes in ascorbic acid content (mg/100gm fruit pulp) of healthy and infected fruits of pineapple, snake gourd and caramobla.

Fruit/ Vegetable	Ascorbic acid Content				%loss in asçorbic acid after 12 days of incubation.
	Days of Incubation				
	0	4	8	12	
<u>Pineapple</u>					
Healthy	43.5	36.2	31.1	21.2	51.2
Infected	43.5	32.3	19.8	8.5	80.4
(*A. alternata*)					
<u>Snake gourd</u>					
Healthy	26.3	22.1	18.1	11.6	55.8
Infected	26.3	15.7	10.6	4.5	82.8
(*F. oxysporum*)					
<u>Carambola</u>					
Healthy	28.7	23.2	17.3	10.3	64.1
Infected	28.7	16.5	12.3	4.5	84.3
(*B. theobromae*)					

percentage loss of ascorbic acid in carambola infected with B. theobromae was 84.3%, while in the healthy one it was 64.1%.

According to **Tandon** (1970), "The loss of Vitamin C under pathogenesis may be due to the enzymes either by the fungus alone or by the host-pathogen complex".

The pronounced decrease in ascorbic acid content of healthy as well as diseased fruits of pineapple, snake gourd and tomato after 12 days of incubation period may be due to the increased rate of respiration during pathogenesis caused by the pathogens or due to the activities of ascorbic acid oxidase.

CONTROL MEASURES

The phytopathologists are the trained plant doctors, the medicine men of agriculture, whose final goal is successfully to prevent or control crop disease

[F.D. Heald, 1926]

Plant diseases play an important role in determining the amount and cost of food. Plant pathology must alleviate the food problem by devising new control measures and improving the older ones. The annual loss to World crops as a result of disease has been estimated at 25,000 million dollar; of this a major part is due to fungal pathogens. Surface deposits of fungicides can control many fungal diseases. There is an enormous amount of literature available on chemical used for plant disease control.

The use of chemical sprays, dusts or seed treatment for protecting plants from the ravages of the pathogen is not an innovation of the 20th century. The early agriculturists did try to control plant diseases by utilizing chemicals and fungicides.

Chemicals in disease control have been traced far back into history. The first recorded mentioned of plant disease control is in the writings of the Greek poet, **Homer** (1000B.C.), who mentions sulphur, which is still in use. Also, the fumigation of trees with Bitumen and sulphur was mentioned by the Roman patriot, **Cato** (200 B.C.). The first landmark in the control of fungal diseases of plants was the discovery by **Anton de Bary** that the causal agents of many plant diseases are fungi. The development of fungicides rapidly followed this discovery. It is after this period that **Pierre Alexis Millardet** (1885) showed that the downy mildew of grapes could be controlled by mixtures of copper sulphate and lime. Copper sulphate had been in use even before that but it was only in 1885 that its toxicity were reduced by mixing it with lime.

The object of the plant disease control is to prevent economic loss and increase the value of the crop. Complete eradication of the pathogen is secondary and only a means of achieving this object. In attempting to check diseases of plants, knowledge

is usually required of the cause of the disease, of the life history of the parasite, and of the circumstances which influence the establishment of parasitic relations between it and the host.

Special problems and special opportunities arise when chemicals are applied under post harvest circumstances. That is, chemical of post harvest troubles is more typically an auxillary than a principal approach, taking second place to temperature control, atmospheric monitoring, moisture control, and so on.

Even so, chemicals are a not unimportant means to the desired end, although there are a number of difficulties. For edible products the question to toxic residues becomes of immediate concern and rules out a substantial number of compounds that might otherwise be suitable. Time of application whatever the substance chosen, must take into account the biology of host and pathogen: whether infection stems mostly from an earlier, preharvest condition, or whether it is directly related to the storage and handling phase. Some accommodations must be made for

the span of time over which the produce in question is vulnerable to the pathogen or pathogens involved. And it is necessary to fit the control measures economically and logistically into the special characteristics of the produce involved, the customary handling methods, the storage facilities and duration of time in storage and eventual sale and use of the commodity.

Over the years an arraying of compounds has been used. First were the chemical washes or dipsrather nonspecific antiseptics such as borax, hypochlorite, and sodium carbonate. Gradually more effective and More specific compounds were developed and brought into use-sodium O-phenylphenate, dithiocarbomates, and a number of 'organics' (2,6-dichloro-4-nitroaniline, captan, thiabendazole, and others).

Before discussing the various chemicals for plant disease control, it is essential to define a fungicide. A fungicide may be defined as, "any agent, chemical or otherwise, which is applied to the plants, their parts or their environment for purpose of

disinfection or protection from disease-producing organisms". In other words we can say that a fungicide is a agent that kills or inhibits the development of the fungus spore or mycelium. they may be in liquid, dust, or gaseous form. The fungicides used on plants may be classified as protectants, eradicants and therapeutants on the basis of their uptake by and mobility within plant tissues.

Apart from being useful to plants, the fungicides have some negative aspects too. On the matter of toxicity to man and crops, controversy has raged back and forth for a long time. Disease control chemicals are poisons, designed to kill or at least seriously disadvantage pathogenic organisms. As such they are likely to be toxic to other organisms with which they come into contact. This effect is most frequently encountered as phytotoxicity when, by reason of unwise tuning, incorrect preparation, or poor choice of materials, the host is directly injured by the chemical used. Toxicity to man shows up in three ways. First are the routine hazards of inhalation, ingestion, or absorption through the skin

during otherwise careful application. As second is the danger, as with any other toxic substance, of accidental poisoning through mistaking the stored pesticides for an innocuous substance. Most difficult to assess, and therefore most controversial, are the hazards represented by pesticide residues on plants and plant parts used for food or that accumulate in crops and livestock situated on land that has in it deposits of one or more recognized agricultural poisons. The use of toxic chemicals are pernicious and come uneffective on the pathogens.

Moreover, by the use of chemical ,pathogen controller, upsetting the biological balance can lead to severe outbreaks of disease. Now the public wants cheap and rapid biocontrol of pathogen and diseases. There is thus, evident and renewed increased interest in biological control of plant pathogens.

The role of biological agents in the balancing of plant and animal life is as old as nature but the use of such agents in the control of plant and animal diseases on the part of man is something comparatively recent. There are two ways in which a biological agent

may be useful in the control of disease. First by directly attacking the organism causing the disease and second, by secreting an antibiotic substance which indirectly reduces the activity of the disease pr ducing organism.

Antibiotics are substances which are produced by microorganisms and which act against microorganisms. Most antibiotics known uptil now are products of actinomycetes and some are from fungi and bacteria. The chemical nature of antibiotics is complete and is not, as a rule related to each other. A large number of antibiotics have been tried for plant disease control but only a few have been successfully utilized. The important antibiotics for the control of plant diseases are streptomycin, teracyclines, griseofulvin, cycloheximide and aureofungin. The use of antibiotics is a comparatively new method of plant disease control, but antibiosis or atagonistic phenomena among micro organisms and their relation to plant diseases have been recognized for more than 75 years. Giliotoxin, the first antibiotic to be used for plant disease control, was isolated and

purified by a plant pathologist even before the discovery of penicillin.

A number of cases have been recorded in which antibiotic substances are known to be secreted by organisms which have a definitely toxic effect upon some of the disease-producing organisms. Substances like the penicillin and streptomycin are examples.

Alexopoulos found that cultures of Actinomyces albus inhibited all fungi against which it was used. Among the fungi which it inhibited were Glomerella cingulata, Physalospora cydoniae, Gloeosporium roseum, Colletotrichum lindemuthianum. The host plant itself may secrete an inhibitory substance which is toxic to the pathogen. **Berridge** found that the sap of potatoes contained a substance which possessed some agglutinating power over the cells of Bacterium tumefaciens, B. solanasaprus and B. phytopthorus. **Thomas** found that the galls of the olive, formed as a result of the attack of Bacterium oleae, contain a substance which well inhabit the growth of the bacteria.

Bajaj and **Gosh** (1975) in an article on antifungal antibiotics have made the following observation : "The advent of an antibiotic era had raised the hope that the antibiotics would provide us with the much awaited chemotherapeutants effective in plant disease control As far as fungal diseases are concerned, this expectation has not been fulfilled to a significant level. This fact will be evident when the plethora of reports on new antibiotics effective in vitro against numerous phytopathogenic fungi, that had been pouring in for the past three decades, is compared with the meager number of antibiotics which have found any real application in plant disease control under field conditions tell today" **Zaumeyer** (1958) reviewed the antibiotics used fore controlling plant diseases. Their uptake and tranlocation have been discussed by **Goodman** (1959), **Crowdy** (1959), **Crowdy** et. al. (1955, 1956), **Pramer** (1959) and **Dimond** and **Horsfall** (1959). Further useful information has been given by **Dekker** (1963, 1969). There are special reviews on cycloheximide by **Ford** et. al. (1958) and on griseofulvin by **Brain** (1960). An article on the

development of agricultural antibiotics is by **Misato** (1977).

The main purpose of control to plant diseases, is first, to check the economic losses and second, to increase the quality and quantity of crop. The uses of fungicides and antibiotics is helpful in this respect. Fungicides and antibiotics are just one of the weapons in our arsenal of defence against the diseases of fruits and vegetables. A fungicide may be toxic to one pathogen, while it may be harmless to another.

According to **Lilly** and **Barnett** (1951), "There is no universal fungicide." Therefore, it becomes necessary to assess the efficacy and effect of fungicides both on the causal organism and the host in the laboratory before commencing any field trials. **Gutter** (1969), **Eckert** (1969), **Daines** (1970), **Wells** and **Harvey** (1970), **Wells** (1972), **Bolakan** et al. (1976), **Dekker** (1977), **Darvis** (1978 and 1982), **Hall** (1983), **Glazener** and **Covey** (1984) and many others have made some outstanding contributions in this field.

In the present investigation the following six fungicides and two antibiotics were used against *A. alternata*, *F. oxysporum*, and *B. theobromae* :

(a) Fungicides :

1.	Blitox	50% Copper oxychloride
2.	Dithane M-45	Zinc ion and manganese ethylene bisdithiocarbamate.
3.	Bavistin	Carbendazin 2-(methoxy-carbomoyl) benzimidazole.
4.	Blue Copper 50	88% (W/W) Copper oxychloride.
5.	Captan	1,2,3,6-tetrahydro-N-(trichloromethyl thio) phthalimide.
6.	Tecto-40	42.28% 2-(4-thiozolyl)-benzimidazole.

(b) Antibiotics :

7.	Mycostatin (nystatin or fungicidin)	A preparation of antifungal antibiotic nystatin present in the mycelium of *Streptomyces noursei* (**Brown** and **Hazen**, 1957).

8.	Idifulvin (griseofulvin)	7-chloro-4,dimethoxy coumaran - 3-one - 2 -Spiro-1'-(2' methoxy 6'- methyl cyclohex-2'-in - 4'-one).

Evaluation of fungicides and antibiotics *in vitro*:

For the determination of the most effective concentration, poisoned food technique as described by **Nene** and Thapliyal, 1979, pp. 413-414 was used in which required amount of each fungicide was mixed with the known amount of sterilized basal medium before solidification and then was poured in the petri dishes. The three pathogens under investigation were inoculated and their growth was recorded after 7 days of incubation. Absence of fungal growth at any concentration gave the minimum concentration required to control the pathogen *in vitro*. The results are summarized in table —11.

Table-11:- Effect of different concentrations of fungicides/antibiotics on the growth of three pathogens under investigation.

Fungicides/ Antibiotics	Concentration in ppm	*A.alternata*	*F. oxysporum*	*B. theobromae*
Blitox	100	+	+	+
	250	+	+	+
	500	+	+	+
	750	−	+	+
	1000	−	−	−
Dithane M-45	100	−	+	+
	250	−	+	−
	500	−	−	−
	750	−	−	−
	1000	−	−	−
Bavistin	100	−	+	−
	250	−	−	−
	500	−	−	−
	750	−	−	−
	1000	−	−	−
Blue Copper 50	100	+	+	+
	250	+	+	+
	500	−	+	−
	750	−	−	−
	1000	−	−	−
Captan	100	+	+	+
	250	+	+	+
	500	+	+	−
	750	−	−	−
	1000	−	−	−
Tecto-40	100	−	−	−
	250	−	−	−
	500	−	−	−
	750	−	−	−
	1000	−	−	−

Mycostatin	25	+	+	+
	50	+	+	+
	100	-	-	-
	250	-	-	-
	500	-	-	-
Idifulvin	25	+	+	+
	50	+	+	+
	100	+	+	+
	250	+	+	+
	500	-	-	-

\+ Shows presence of the fungal growth.
\- Shows the absence of the fungal growth.

It is clear from the table —11, that all the six fungicides and both the antibiotics were effective against the pathogens under study at different concentrations. Blitox was effective at 750ppm concentration for A. alternata, while it was effective at 1000 ppm for F. oxysporum and B. theobromae. Dithane M-45 was effective agaisnt the growth of A. alternata at 100ppm, while it was effective for B. theobromae at 250ppm and for F. oxysporum at 500ppm. A. alternata and B. theobromae failed to grow at 100 ppm of Bavistin, whereas 250ppm of it was effective for F. oxysporum. Blue copper50 at 500 ppm was inhibitory to A. alternata and B. theobromae, while its effective concentration for F. oxysporum was found to be 750ppm. A. alternata and F.oxysporum failed to

grow at 750ppm of Captan, while 500ppm of Captan was effective against B. theobromae. All the three pathogens failed to grow at 100ppm of Tecto-40.

Mycostatin could check the growth of all the three pathogens under investigation at 100ppm. Idifulvin at 500ppm was effective against the three pathogen.

USE OF FUNGICIDES/ANTIBIOTICS *in vivo*:

Since Blitox was effective only at higher concentrations, so that was not selected for further use. The remaining fungicides and antibiotics were tried in vivo. In addition to their minimum inhibitory concentrations some higher concentrations were also tried in order to achieve maximum disease control. For in vivo studies, both pre and post inoculation treatments were given in the fruits. For pre-inoculation treatments, they were first dipped in known concentration of chemicals for 5 minutes and were allowed to dry for 3-4 hours, thereafter they were inoculated with the fungus. In post-inoculation treatments, fruits were first inoculated with the

pathogen and after a lapse of 24 hrs, they were dipped in different concentrations of fungicides or antibiotics for 5 minutes. In the control series the fruits/vegetables were dipped in sterilized distilled water in place of chemicals. Treated fruits were incubated for 10 days, thereafter the percentage of fruits protected was determined with the help of following formula as suggested by **Lal** et al. (1981).

$$\text{Percentage Control} = \frac{\text{\% decay in control} - \text{\% decay in treatment}}{\text{\% decay in control}} \times 100$$

Where;

% decay = (W - w)/w x 100

W = Weight of the fruits before inoculation.

w = Weight of the fruits after removal of the infected tissue.

The results are given in table —12.

Table-12:- Efficacy of different fungicides and antibiotics in checking (expressed as percentage control) the diseases under investigation.

Fungicides/Antibiotics	Concentration in ppm	Pineapple (*A. alternata*)		Snake gourd (*F. oxysporum*)		Carambola (*B. theobromae*)	
		Pre	Post	Pre	Post	Pre	Post
Dithane M-45	250	2.5	5.2	5.5	4.3	2.1	2.3
	500	16.1	14.2	16.5	12.5	14.6	14.1
	750	46.0	39.2	39.2	32.6	36.5	31.3
Bavistin	100	21.2	18.9	11.7	8.2	10.0	11.9
	250	31.6	29.8	23.8	23.5	29.8	21.5
	500	65.2	52.0	52.6	50.1	63.0	58.2
Blue-Copper	250	5.7	3.1	10.7	7.1	6.4	4.5
	500	21.2	14.8	23.0	21.0	15.2	11.2
	750	45.0	40.5	38.2	32.2	29.1	23.8
Captan	250	5.9	8.5	0.0	0.0	3.9	2.3
	500	23.5	17.3	12.5	9.5	18.2	15.2
	750	39.1	37.0	25.4	22.0	26.4	23.2
Tecto 40	100	8.6	6.3	10.1	14.6	4.8	7.8
	250	20.1	16.8	23.6	20.3	24.1	17.3
	500	49.4	45.3	61.4	58.2	53.2	49.1
Mycostatin	50	5.5	3.8	3.0	3.5	0.0	0.0
	100	10.2	9.8	14.4	12.2	10.6	8.2
	250	26.3	26.1	29.8	25.6	24.0	20.1
Idifulvin	250	3.0	2.8	0.0	0.0	0.0	0.0
	500	11.9	10.2	5.2	6.5	4.8	2.2
	750	19.7	17.5	14.8	12.3	23.2	20.3

It is clear from the table- 12, that none of the fungicides and antibiotics could control the disease completely at any concentration tried. Generally the pre-inoculation treatment was more effective than the post-inoculation treatment. In few cases the reverse was also true, however, no difference was observed in pre-and post-inoculation treatments in some of the cases.

Percentage control of Pineapple infected with A. alternata was maximum at 500ppm of Bavistin followed by Tecto 40 (500ppm), Blue Copper (750ppm), Dithane M45 (750ppm) and Captan (750ppm). Out of two antibiotics, the % control was maximum at 250ppm of Mycostatin follwed by Idifulvin at 750ppm.

Percentage control of Snake gourd infected with F. oxysporum was maximum at 500 ppm of Tecto-40 follwed by Bavistin (500ppm), Dithane M-45(750ppm), Blue Copper (750ppm), Captan (750ppm). Of the two antibiotics tried Mycostatin (500ppm)was more effective. It gives 29.8% and 25.6% control in pre-and post-inoculation treatments respectively.

Out of 6 fungicides used to control the rot of Carambola, Bavistin (500ppm) gave the most satisfactory result. At this concentration 63% and 58.2% disease was controlled in pre- and post-inoculation treatments respectively. Other fungicides in their descending potentialities included Tecto 40 (500ppm), Dithane M-45 (750ppm), Blue Copper (750ppm) and Captan (750ppm). Among antibiotics, the percentage. control of carambola was maximum at 250ppm of Mycostatin followed by Idifulvin at 750ppm.

From the above findings, 500ppm of Bavistin can be recommended for controlling the diseases of pineapple and carambola. The, same concentration (500ppm) may be recommended for controlling the rot of snake gourd. The antibiotics used were also effective but being expensive their use on commercial scale may not be possible.

Besides chemical treatment following precautions can be taken to prevent fruits & vegetables from catching the infection :

(1) **Careful handling** : It has been observed that careless handling is responsible for the rots of storage fruits and vegetables in most of the cases. So to avoid any sort of injury the fruits/vegetables should be dealt carefully during harvesting, transportation, storage and marketing.

(2) **Use of disinfectants** : A proper sorting and cleaning of the fruits/vegetables before storage should be strictly followed. 6-8% borax solution or 250-300ppm of mycostatin may serve as useful disinfectants.

(3) **Exclusion of diseased fruit from the healthy one**: Diseased fruits are the source of inocula. Hence their contact with the healthy ones should be strictly avoided. It can be achieved by regularly inspecting the stored fruits/vegetables.

(4) **Exclusion of infected leaves in the packing**:

Usually the fruits are packed with the leaves of their plants. There are possibilities of

infection through these infected leaves. Therefore, it is very essential to remove these leaves before packing the material.

(5) **Use of Clean Containers** : The produce should not be packed in a previously used containers. They may contain the fragments of rotted tissues which may serve as a source of inoculum. Care should be taken to use sterilized crates for packing the produce.

(6) **Low temperature Storage**: The incidence of fruit/vegetable rots could be minimized by storing the produce at low temperatures i.e. between 50 to 10^0C.

(7) **Quick transport and sale** : Quick transportation and sale of the produce will give less time for the pathogens to start on their destructive course.

(8) **Spray treatments**: The initial check of the disease could be possible by giving spray treatments during blossoming and fruiting.

.........biological control is much neglected, not because it does not work but because not enough research is done on it. Biological control has worked, is working and can, if we device it, greatly extend its successes........

- **R.ORDISH, 1967**

Man slipped gradually into the direct control of plant diseases after he began to use toxic chemicals in the mid-eighteenth century. This seemed the rational thing to do, because fungicides could often be applied after the disease appeared and rapid effective control was provided. The concept of overkill slowly evolved particularly in soil treatments. However unexpected and disturbing results increasingly appeared, indicating that there was more to soil treatment than merely killing microorganisms (**Baker**, in **Toussoun** et. al., 1970). About the same time, insects became increasingly resistant to proprietary insecticides, and soon fungi resistant to fungicides, and bacteria to antibiotics, began to appear. In the 1960s the public became aware of the pollution problem, and of the increasing concentrations of toxic chemicals in nature's food chains. The pendulum of public opinion, once so

favourable to this cheap and rapid control of pests and diseases, now threatens to swing too far in the direction of forbidding use of any chemicals, even to providing organic gardening with a new talking point. Paralleling the public's rediscovery of its environment, plant pathologists are becoming increasingly aware that upsetting the biological balance can lead to severe outbreaks of disease. Thus, there is evident a renewed and increased interest in biological control of plant pathogens. Pathologists are beginning to purposefully involve biological control in their integrated control programs for plant diseases.

The occurrence of a plant disease indicates that some aspect of the biological balance is not in equilibrium, and the greater the imbalance, the more severe the disease is apt to be.

Conversely, absence of disease means that the pathogen is absent, the host is highly resistant, the physical environment is unfavourable part of all of the time, or antagonists are inhibiting the growth or

infection by the pathogen. Biological control may thus be a restoration of a disease-inhibiting balance in nature.

Because of the multiplicity of factors involved in a disease complex, satisfactory control is rarely achieved by a single measure alone. Although fungicide applications are often thought to provide single-shot control, they usually do not.

Slowly the idea developed that man could manipulate one organism or one aspect of the environment in such a way as to control another. The development of the entomologist's concepts and philosophies of natural control (maintenance of a fluctuating population density of an organism within certain limits over a period of time by the combined actions of the total environment) has been outlined by **Doutt**, **Huffaker**, and **Messenger** (in **De Bach**, 1964). Doutt cites the introduction of the mynah bird from India to Mauritius in 1762 to control the red locust as the first successful transfer of a natural enemy from one country to another. The first really

successful control of an insect by another introduced species was by the vedalia beetle in California in 1888 on the cottony-cushion scale of citrus.

Such control of insects was referred to as parasitic control, bug vs. bug, use of insect enemies, and nature's remedies. In 1916, **L.O. Howard** referred to it as "the biological method", and in 1919 **H.S. Smith** called it "biological control" (**De Bach**, 1964.

A unified concept of biological control may be stated as :

"Biological control is the reduction of inoculum density or disease - producing activities of a pathogen or parasite in its active or dormant state, by one or more organisms, accomplished naturally or through manipulation f the environment, host, or antagonist, or by mass introduction of one or more antagonists."

This definition may be paraphrased for 2 specific examples : Biological control of pine-stump infection in the reduction of disease-producing

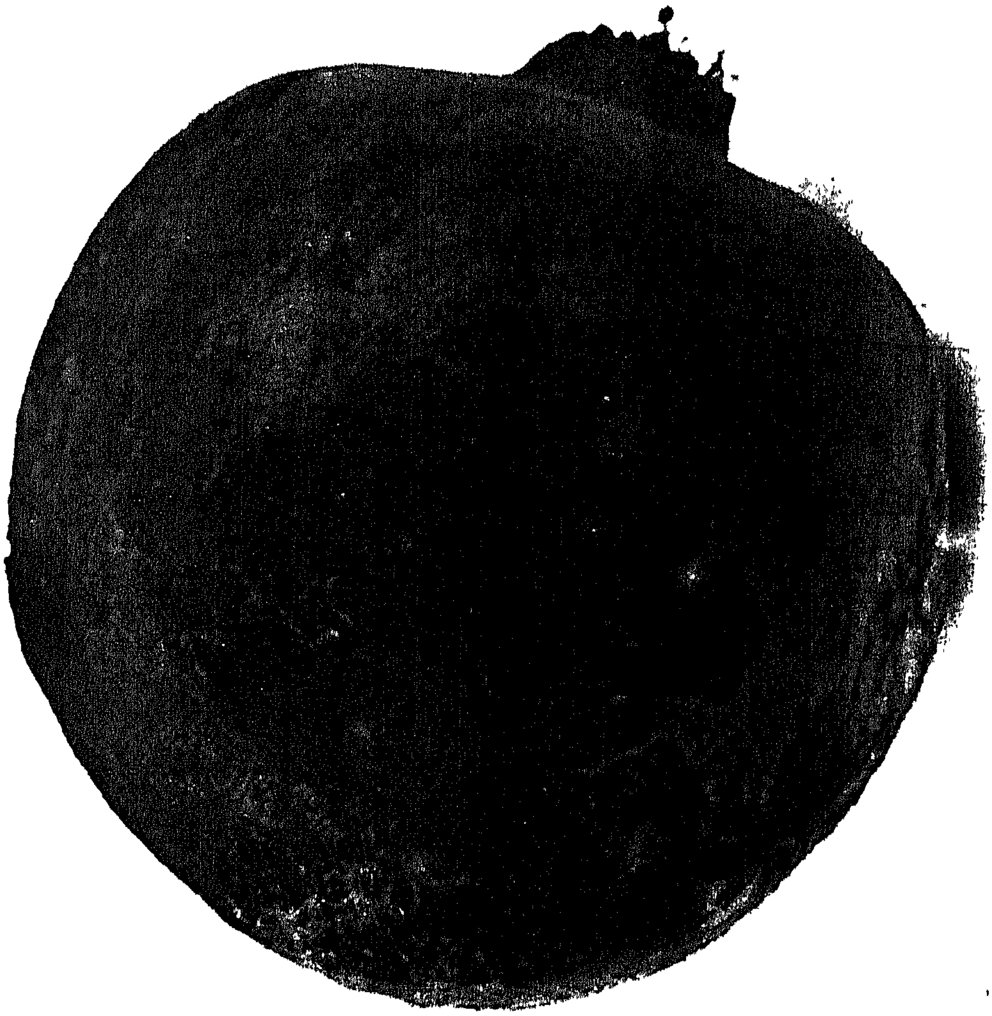
activities of Fomes annosus in its active state, by Peniophora gigantea, accomplished through competition from the mass introduction of the antagonist. Biological control of wheat seedling blight is the reduction of the disease-producing activities of Fusarium roseum f. sp. cerealis 'Graminearum' in its active state, by the wheat plant, accomplished through manipulation of soil temperature.

Wood and **Tveit** (1955) were able to control the spread of Fusarium nivale on oats in England. Seed, heavily infested with Fusarium, was sown in the field along with oat straw cultures of various Chaetomium cochliodes isolates. One isolate gave 38-40% stand of plants at harvest, as against 62% for seed treated with an organomercurial dust, and 25% for the check.

Tveit and Moore (1954) found in Minnesota that Chaetomium globosum and C. cochliodes, which occurred naturally on oat seed from Brazil, were the real basis of the supposed resistance of these varieties to Helminthosporium victoriae in the field.

Man is learning that the best answer, to the severe fluctuations in pathogen attack resulting from his use of "dynamite" or overkill treatments is not ever-more-potent treatments that produce successively stronger and more extensive oscillations.

DISCUSSION AND CONCLUSION



Basic research is a creative venture of a high order.
It flows from the minds of a relatively few men
and flowers generally in a climate of
quiet contemplation.

[Anonymous]

Fungi which cause diseases of fruits and vegetables during the post-harvest phase are of considerable importance and it can be stressed that a more thorough probe should be directed towards such diseases. An extensive search reveals that a number of fungi belonging to diverse taxonomic positions may be responsible for the decay and deterioration of various fruits and vegetables. In order to have a proper assessment of the damage caused by the pathogens, it is essential to as to ascertain the relationship which the organism have with their hosts.

Harvested fruits and vegetables are vulnerable to attacks by microorganisms because of their high moisture.content and rich nutrient. Due to harvesting, packing and transportation, injuries of various kinds are caused which facilitate the entry of certain

pathogens. A single infected fruit can also be the source of infection to other fruits and vegetables during storage and transit.

Investigations carried out by various workers clearly indicate that the real causes of the spoilage of fruits in transit and storage are the high temperatures and injuries sustained by the fruits during the process of marketing.

High moisture content makes it difficult and expensive to conserve them as dry products. They bruise easily and metabolically active than the durable. These characteristics significantly limit the storage life of vegetables and fruits and post harvest life may, therefore, be only a few days.

High temperature and relative humidity favour the development of post-harvest decay organisms. More acidic tissue is generally attacked by fungi,, while vegetables or fruits having pH above 4.5 are more commonly attacked by bacteria.

Such diseases, which are noticed after the harvest, considerably reduce the commercial value of the produce.

The results obtained in the present investigation have been discussed fully at appropriate places in the different chapters. However, in this chapter only efforts have been made to correlate the results obtained in various experiments and certain conclusions have been drawn where possible.

The ability of fungi under study to infect a wide variety of fruits belonging to diverse taxonomic groups shows their strong parasitic nature. Though the losses in such cases appear during storage but in many cases the organisms responsible for the diseases become associated in the filed. Some of the fungi may, however, attack the fruits during storage. Morphological study of various pathogens recovered from different fruit rots showed that there was some variation in the morphology of certain isolates of the same species. This night be due to different types of nutrition they get form the different fruit. It has

been realized by phytopathologistis that it is essential to study the factors facilitating the growth and spread of the diseases by the pathogenic fungi. In the chapter five these factors are discussed in detail.

Pathological studies of the three pathogens under investigation indicated that they were pathogenic on their respective host and were also capable of infecting a wide variety of other fruits. This may help their survival in nature. A detailed study of pathogens causing rots of different fruits and vegetables has been given in "Isolation studies". Also, different rots caused by different pathogens have been described in detail under "Pathological studies". These studies clearly indicates that a single pathogen is capable of infecting a wide range of hosts.

The study of the spores of the pathogens included the mode of germination of the spores and also how some external factors affect their germination. All the three pathogens under

investigation absorb water within ½ an hour so that their protoplasmic contents became visibly distinct. However, their time of germination differs i.e. between 3-5 hours. The germ tubes of A. alternata appeared after about 3½ hrs., F.oxysporum between 4-5 hours and B. theobromae after 2½ hours. Effect of nutrient solutions showed that the maximum spore germination was observed in host decoction followed by basal medium. The spore germination was poor in distilled water due to the lack of nutrient in it. Also tap water showed minimum germination, probably due to the presence of chlorine. Good percentage of germination of spores of the three fungi under study on host decoction also established the suitability of the hosts for the pathogens.

Similarly the effect of different temperature on spores germination was observed. The spores were found to germinate between 10^0C-35^0C, but the maximum germination was recorded at 25^0C. At this temperature the growth and the sporulation of the pathogens were also maximum. There was no spore germination a 5^0C and 40^0C temperatures.

The study of spores germination at different pH levels showed that the pH value of 6.0 was most suitable for the germination of spores of all the three pathogens under investigation. However, the spores failed to germinate at pH 2.0 and 10.0. pH range above 6.0 showed the gradual decline, whereas, there was an increase in germination between 3.0 to 6.0. This versatality of the organisms enable them to survive at the expense of the host even in varying pH conditions. These studies further proved that the pathogens were capable to survive even under the unfavourable conditions. They were capable of surviving well in the intense heat. A high thermal death point reflected their capability to tide over the high temperatures. It was found to be between 55^{0}C to 60^{0}C. Usually the thermal death point of the fungi lie between 40^{0}C to 60^{0}C.

Carbohydrates serve as exclusive source of energy. They have special significance in the carbon nutrition of pathogenic fungi. Glucose, fructose, sucrose and starch are usually present in most of the

fruits. They were detected in the healthy hosts under study.

Chromatographic analysis of healthy and infected tissues of various fruits in respect to their amino acid, organic acid and sugar contents as well as a study of post infection changes in their ascorbic acid content revealed that the infection by the pathogens not only disfigured the fruits externally but also cause marked changes in the nutritional complex of the hosts.

After getting entry inside the fruit, the pathogen brought about considerable changes in free as well as protein bound amino acids and utilized many of them which in turn entered into the amino acid pool of the fungus occasionally the concentration of some amino acids increased in the infected tissues which may probably be due to the proteolysis of the host protein catalysed by the fungal enzymes or by the host enzymes. A number of investigations were carried out including the work of **Kapoor** and **Tandon** (1969a), **Bhargava** and **Tandon** (1976), **Thind** et al. (1977) and

Tewari (1986). They studied the changes in the organic acid contents of certain fruits during the pathogenesis. Changes in the sugar contents of diseased fruits by various fungal pathogens have been studied by many workers including **Singh** and **Tandon** (1970), **Chahal** and **Grover** (1972), **Grewal** and **Grover** (1974), **Sharma** and **Wahab** (1975), **Bhargava** and **Tandon** (1976), **Singh** and **Chauhan** (1977), **Rai** (1982), **Tewari** (1986) and **Malviya** (1992). **Ghosh** et al. (1966), **Srivastava** and **Tandon** (1966b), **Singh** (1968), **Tandon** (1970), **Pandey** (1983), **Tewari** (1986) and **Malviya** (1992) have showed that fungal decay causes huge loss in ascorbic acid content of healthy and diseased fruits.

During pathogenesis the organic acid contents of the fruits either increased or decreased. Some were completely utilized by the pathogens. Appearance of few organic acids in the diseased fruits was also recorded.

A study of post infection change in ascorbic acid content of different fruits showed that the

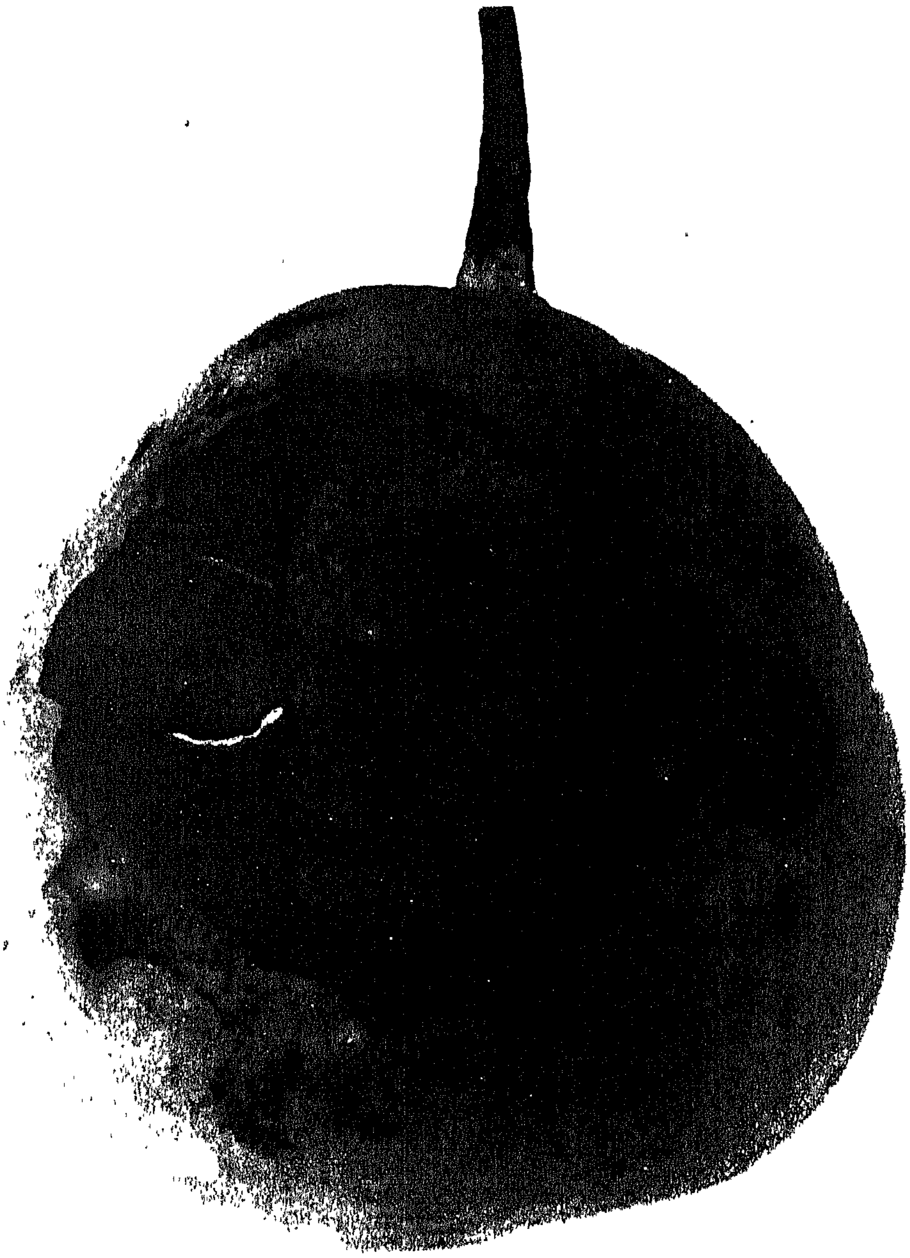
quantity of this vitamin decreased in both the healthy and infected fruits with the increase of the incubation period. However, the losses were comparatively greater in diseased fruits than in healthy ones.

Studies on evaluation of different fungicides and antibiotics indicated that 250 ppm. of Bavistin and 100 ppm. of Tecto-40 was effective against all the three pathogens. Other chemicals were also effective but at higher concentrations. Other fungicides in their descending potentialities are Dithane M-45, Blue Copper, and Captan, all at 750 ppm. among the antibiotics, mycostatin was more effective as compared to idifulvin. But being costlier their use on commercial scale will not be economical. Since Blitox was effective at higher concentration, so it was discarded.

It may be concluded from the above studies that the fungi under study varied considerably in their nutritional and pathogenic behaviour and a close host-parasite relationship was established in each case.

Thus, it is very important that a detailed study of pathogens must be carried out for proper understanding of the behaviour of pathogens responsible for causing diseases.

SUMMARY



Our research must be good, but it must be good for something.

[Norman Borlaug]

The purpose of this thesis is to summarize and organize our knowledge of the "Pathological studies of some fruits and vegetables infected by fungi in markets".

Plant production is essential to the maintenance of life of man and animals upon this planet. Food, clothing, many luxuries and some essential drugs are dependent directly or indirectly upon growing plants.

India provides suitable conditions for development and spread of numerous plant diseases because it is a subtropical country with warm and humid climate. Disease losses are hazards which can be minimized only by a continuous process of research and education. Fungi consists of large number of parasitic and saprophytic organisms, well equipped to complete their life cycles within a very short span and are

well adapt to the changing environment. They not only inhabit almost all parts of the plant before harvest but also deteriorate fruits and seeds during post harvest phase.

The study of fungi was diligently pursued by several able systematists like **Bulliard**, **Batsch**, **Persoon**, **Link**, **Schweinitz** and **Fries**.

Curiosity demanded as to what these fungi were and where did they come from **Pliny**, a Roman naturalist (about 3000 B.C.) could not decide whether fungi were living or non-living. The common belief was that they originated from thunder of clouds and a fluid accompanying the thunders penetrated the earth and gave rise to fungi (**Buller** 1915). The first illustrations of microfungi were made by **Robert Hooke** (1635-1703) in his book "Micrographia" (1665). **Hooke** drew the sporangia of Mucor and the teliospores of the rose-rust Phargmidium micronatum. **P.A. Micheli** made an extensive study of the fungi and their reproductive structures. **Oscar Brefeld** (1839-1925) studied fungi

by growing them in pure culture under varying conditions.

Since a number of important diseases have not been studied, since the effective control measures of several diseases have still not been worked out. A country like India battling on the food front loses approximately 18% of its total production or Rs. 5,000 crores annually due to weeds, diseases and pests (**Mehta**, 1976). Post harvest diseases of fruits and vegetables are responsible for 20-30% loss of the crops annually (**Mehta**, 1975).

So it was considered desirable to made an extensive survey of fungal diseases of fruits and vegetables after harvest in Allahabad and its adjoining areas. The entire work in this thesis are fully elucidated and has been divided into ten chapters in all.

The first chapter deals with the importance of vegetables and fruits, their food value and their diseases. The second chapter gives the information regarding the materials and methods used in the

present investigation. During the investigation period, a good number of fungi were isolated from different hosts which have been described in detail in the third chapter. The fourth chapter provides the information of symptoms produced by different pathogens on their respective hosts. It also contains the study of spores and effect of different external factors on their germination. In the fifth chapter the environmental effect on the development of disease is discussed in great detail. The chapter six deals with the economic losses as well as the nutrients losses due to pathogenesis. The control of diseases and to suppress the growth of pathogens under study are given in chapter seven. The results and conclusions have been discussed in the eighth chapter of this thesis. In the end the reference or literature cited during the work is given in the form of bibliography.

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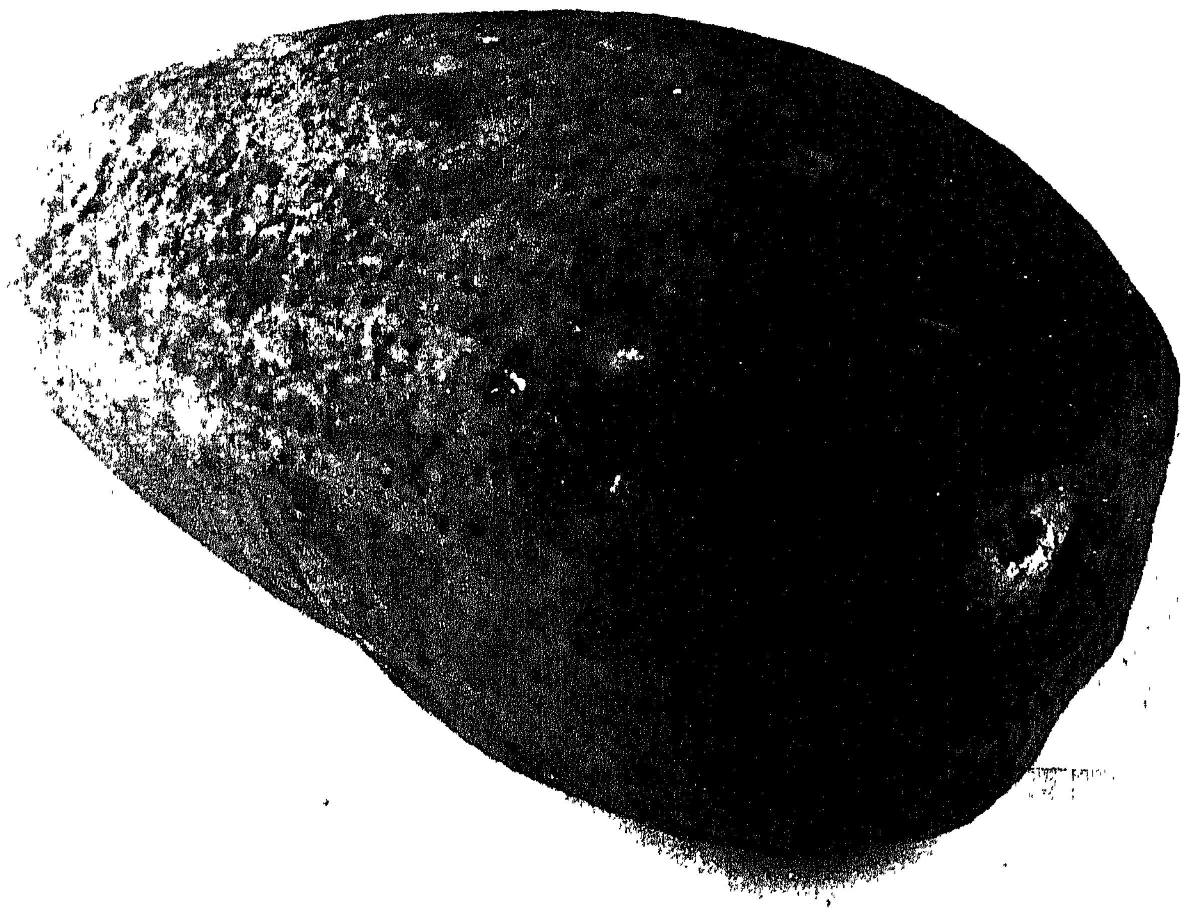

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